MODELING AND CONTROL OF THE ANAEROBIC DIGESTION PROCESS

By

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Anaerobic digestion is a biochemical process that is used for treatment of industrial and agricultural wastes and sewage sludge. Its advantages over aerobic treatment processes are that it does not require aeration, produces low amounts of sludge and generates methane which is a fuel. A major disadvantage of this process is its susceptibility to failure when exposed to disturbances in the form of a feed overloading or inhibitors entering with the feed.

Anaerobic digestion process models can aid in predicting the dynamics of the process when it is subjected to disturbances and in addition aid in designing anaerobic digesters and assessing proper operating strategies. A dynamic model for a glucose fed continuous anaerobic digester is presented. The model incorporates the most recent advances made in the study of this process. The kinetics of the

important metabolic intermediate, pyruvic acid, are included in the model to enhance its predictions when the process is subjected to a feed overload.

An inhibitor entering with the feed, would cause a depression of the maximum specific growth rate of the bacterial populations. If the maximum specific growth rate drops below the dilution rate then washout occurs. By using a simple model for the anaerobic digestion process and employing Pontryagin's maximum principle, an optimal control law that maximizes the total of methane produced over a period of several time constants was obtained. It was shown that this control law was approximated extremely well by a linear relationship between dilution rate and methane rate (constant yield control law). This linear relationship can be easily implemented on an anaerobic digester.

An expert system to prevent failure of the process when it is exposed to disturbances was developed. The constant yield control law was built into this expert system. The expert system uses only methane rate measurements to identify onset of the disturbance. This expert system was implemented on a bench scale anaerobic digester and its efficacy in preventing imbalance when the digester was exposed to a triple overload and phenol, was tested. The expert system successfully prevented digester imbalance.

CHAPTER 1
INTRODUCTION

Objective

The objective of this work is to develop an on-line automatic control strategy to prevent and mitigate imbalance in the anaerobic digestion process.

Technical Perspective

Anaerobic Digestion

Anaerobic digestion is a natural and widely used process for the stabilization of organic matter. It is useful for stabilization because most of the degradable component of the organic matter is converted to methane. This conversion process is carried out by several populations of microorganisms. Almost all natural and many synthetic organic compounds can be stabilized by anaerobic processes. Anaerobic treatment is applied to agricultural wastes like animal manures and crop residues, industrial waste effluents like food processing wastes, brewery wastes and paper and pulp mill wastes and sewage sludge. Degradation of some hazardous wastes can also be accomplished through this process. Several investigators have studied the viability

of using the process specifically for producing energy from plant biomass (Chynoweth and Isaacson, 1987; Smith *et al.*, 1988).

There are several advantages of anaerobic digestion over the conventional aerobic biological treatment processes. In aerobic treatment the waste is mixed with air. The microorganisms obtain energy for growth through oxidation of the organic matter in waste with oxygen. Since these organisms obtain much of their energy from this oxidation, their growth is rapid and a large portion of the organic waste is converted to new cells. Hence, the organic portion is just changed in form (with very little oxidation) and this biological sludge poses a significant disposal problem. However, in anaerobic treatment systems, since air is excluded, the microorganisms have less energy available for growth. Hence, only a small portion is converted to new cells. A major portion of the degradable waste is converted to methane, thus a higher degree of waste stabilization is achieved. As much as 80 to 90 percent of the degradable organic portion of waste is converted to methane. In addition, the requirements of nutrients like nitrogen and phosphorous are greatly reduced enabling the process to be applied for treatment of certain industrial wastes that are deficient in these nutrients. In anaerobic treatment substrates like phenol and other aromatic compounds are broken into smaller molecules, whereas in aerobic treatment these substrates are converted chemically into polymeric adducts which resist further degradation (Schink, 1988). Moreover, since the process does not require oxygen, the treatment rates are not limited by oxygen transfer and no energy is expended for purposes of aeration.

Imbalance Problem

Causes. The anaerobic digestion process is prone to imbalance when exposed to disturbances such as feed overload where the substrate concentration in the feed increases, feed underload where the substrate concentration in the feed decreases, or an inhibitor entering with the feed. Stable performance is based on a symbiotic relationship between various bacterial populations (mainly the acidogenic bacteria, acetogenic bacteria, acetoclastic methane bacteria, and hydrogen-utilizing methane bacteria). An inhibition of any one population can introduce an imbalance in this symbiotic relationship.

Consequences. An imbalance of the process can lead to digester failure. Since the methanogenic bacteria are very sensitive to disturbances, these are inhibited easily. The acidogenic bacteria continue to produce volatile organic acids and these are no longer consumed by the methanogens. Hence, there is an accumulation of these organic acids leading to a "stuck" digester. At this point the digester can no longer process any waste. It may require months to recover a stuck digester causing lengthy shut down of the operation.

Measurements. Traditionally volatile organic acid, pH and alkalinity measurements are used to identify an imbalance (Duarte and Anderson, 1982). When the digester is imbalanced volatile organic acids accumulate resulting in a drop in the pH. However, recent knowledge of the process indicates that volatile organic acid accumulation is a result of an imbalance and not the cause of the imbalance. Gas production rate measurements have also been used to detect imbalance

(Podruzny and van den Berg, 1984). Some researchers (Whitmore and Lloyd, 1986) have used liquid phase hydrogen concentrations to identify the onset of imbalance. Mosey and Fernandes (1989) used gas phase hydrogen concentrations to detect both feed overloads and inhibitors. In this study the methane production rate measurements are used to recognize an imbalance in the process. Most recently it has been shown that on-line NADH fluorescence measurements can be used to detect imbalance (Owens *et al.*, 1991).

Corrective measures. At present very few industrial scale digesters are equipped with automatic control systems that can recognize imbalance and are capable of taking corrective measures immediately. The automatic control systems that exist on industrial scale digesters rely on volatile organic acid and pH measurements to realize the onset of an imbalance (Rusell *et al.*, 1985). A pump is turned on to feed a base so as to bring the pH back to normal levels. Then the cause of the imbalance is identified. If it is a feed overload, the feed is diluted or if it is an inhibitor in the feed then once again the feed is diluted so as to bring the inhibitor concentration below levels that can cause inhibition.

However, several control schemes have been developed for bench and pilot scale digesters using gas (Podruzny and van den Berg, 1984), alkalinity (Rozzi *et al.*, 1985), liquid phase hydrogen (Whitmore and Lloyd, 1986, Dochain *et al.*, 1991), pH (Denac *et al.*, 1988) and digester substrate measurements (Renard *et al.*, 1988). All these schemes involve keeping the measured value at a set point by manipulating the

feed rate. The set point is the value of the measured variable during normal digester operation.

Limitations. Accumulation of volatile organic acids and the associated pH depression are the end results of an imbalance, not the cause. Hence by the time these changes are detected, it might be too late to take any corrective action. Gas production rate is not a reliable indicator of imbalance. Response of the gas production rate to inhibitors is inconsistent because it depends on the type of toxicant. If the toxicant inhibits the growth rate of the acidogens and methanogens then gas production rate would drop. However, if it inhibits only methanogens then gas production rate increases because the carbon dioxide consumption rate by the methanogens is decreased. Measurement of liquid phase hydrogen concentrations for purposes of digester control is an attractive concept, but hydrogen measurement can pose problems. The concentration of hydrogen in the liquid phase tend to be around a tenth of ppm making it extremely difficult to detect and measurement devices for hydrogen are very expensive.

Automatic digester control schemes that have been developed so far have aimed at preventing imbalance resulting from a feed overload. A common problem associated with treatment of wastes the presence of toxic substances in the waste. These inhibitors appear intermittently making it very difficult to detect before hand. If it is a persistent problem then unit operations can be designed to remove the inhibitor from the feed before it is fed to the digester. The pulses of inhibitor that are fed to the digester can be very toxic to the microorganisms causing an imbalance

in the process. Hence, there is a very urgent need for a control scheme that would prevent an imbalance to the process in the presence of inhibitors in the feed.

Proposed Approach

An expert system was developed here that would prevent imbalance to a digester when it is exposed to either a feed overload or an inhibitor entering with the feed. This expert system makes decisions based on methane production rate measurements. Gas production rate is measured on-line after scrubbing the carbon dioxide. Since a feed underload or an inhibitor entering with the feed can cause a depression in methane production rate, the methane production data are evaluated using statistical tools to distinguish between the two cases. Depending on the cause for a potential imbalance the expert system chooses appropriate control scheme. The complete control strategy can be automated hence eliminating any need for intervention by the operator.

The anaerobic digestion process was modelled by using the most recent knowledge available for this process. A simplified version of the model was used to arrive at a control strategy to prevent imbalance due to inhibitors entering with feed. Then the expert system was developed. Simulations were done using the model to test the expert system. After ensuring that the expert system performed to the desired expectation levels, it was implemented on a bench scale digester.

Subsequent chapters detail the development of the model, theoretical development of a control law to prevent imbalance caused by inhibitors in feed and

the development and testing of the expert system. An important observation regarding the reliability of using volatile organic acid accumulation to identify imbalance is included as a chapter. The final chapter lists the conclusions and recommendations for future work.

CHAPTER 2
DYNAMIC MODELING OF GLUCOSE FED CONTINUOUSLY STIRRED TANK ANAEROBIC DIGESTERS

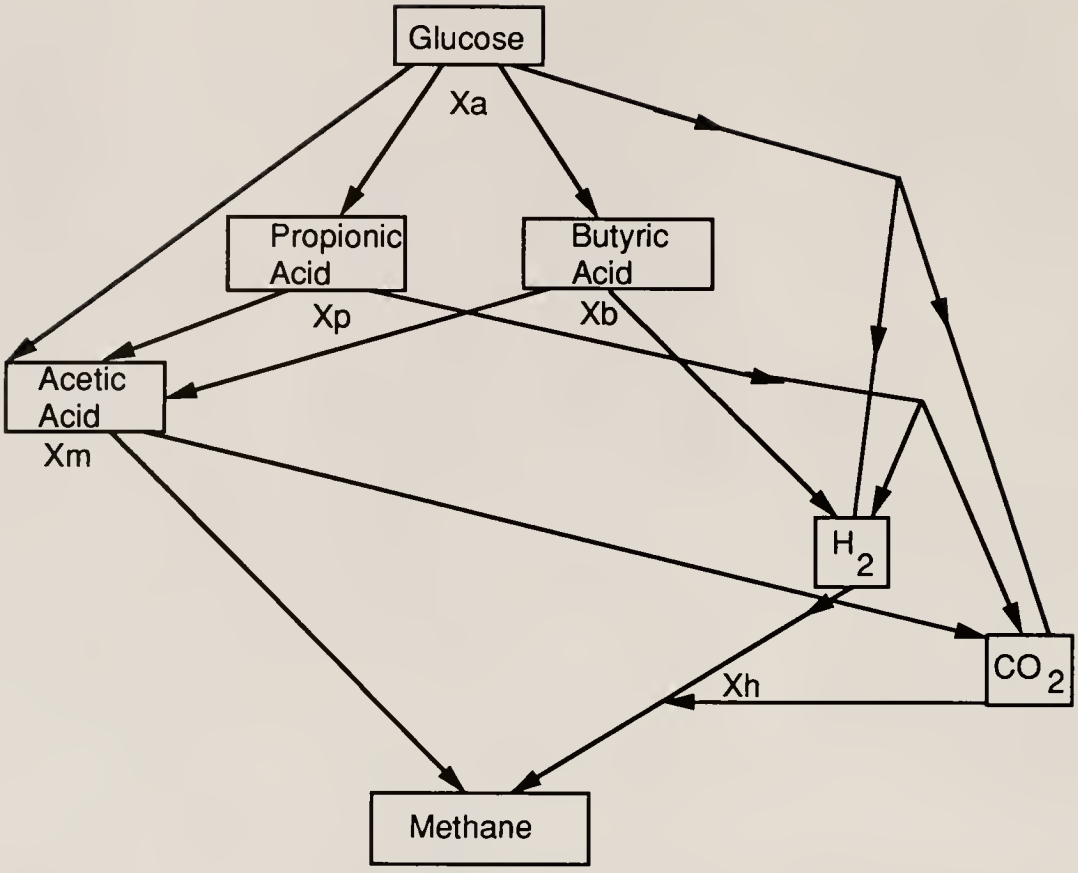
Introduction

Anaerobic digestion process models can aid in predicting the dynamics of the process when it is subjected to disturbances, in assessing proper operating strategies and in designing anaerobic digesters. Such a model must adequately describe the key microbial processes and account for the key variables of interest.

The anaerobic conversion of organic matter into methane involves the concerted action of at least five different groups of bacteria (Zeikus, 1981; Mosey, 1983). The complex carbohydrates, fats and proteins in the waste stream are hydrolyzed by hydrolytic enzymes into simple sugars like glucose. In this work, glucose is used as the major feed substrate; hence the hydrolysis step is not accounted for. The acidogenic bacteria convert glucose to volatile organic acids (primarily acetic, propionic and butyric acids). The acetogenic bacteria utilize the higher acids, namely propionic and butyric acids, producing acetate and hydrogen. There are two classes of acetogenic bacteria, the propionate utilizing acetogenic bacteria convert propionic acid to acetic acid and the butyrate-utilizing acetogenic bacteria convert butyric acid to acetic acid. The acetoclastic methane bacteria utilize

acetic acid as a substrate for growth, producing methane and carbon dioxide as byproducts. The hydrogen and carbon dioxide that are produced in the earlier steps are utilized by another class of bacteria, the hydrogen utilizing methane bacteria, to form methane. It is believed that in healthy digesters approximately 70 percent of the methane produced is due to the acetoclastic methanogens and the rest due to the hydrogen utilizers (Kaspar and Wuhrmann, 1978). The microbial ecology of the process, starting with glucose as the feed substrate, is depicted in Figure 2-1.

Significant work in model development has been done by several researchers. Andrews (1969) and Andrews and Graef (1970) assumed that the methanogenesis from acetate is the rate-limiting step and therefore the most important one in the process. Consequently, they modeled only this step. This effort was followed by more complex models incorporating, most or all of the main bioconversion steps (Heyes and Hall, 1981; Mosey, 1983; Bryers, 1985; Smith *et al.,* 1988; Costello *et al.,* 1991a; 1991b; Pullammanappallil *et al.*, 1991). An important issue is how to model the generation of the different volatile organic acids produced in the acidogenesis step. Smith *et al.* (1988) assumed that the volatile organic acids were generated at a fixed ratio and took into consideration the kinetics of all the steps except that of methanogenesis from hydrogen. Moreover, Smith *et al.* (1988) developed physico-chemical relationships for the liquid phase and state equations, based on mass balances, for the partial pressures of methane and carbon dioxide in the gas phase. These physico-chemical relationships and the state equations enabled the model to predict ion concentrations in the liquid phase, gas composition and gas production



Xa : Acidogenic Bacteria

Xb : Butyrate Utilizing Acetogenic Bacteria

Xp : Propionate Utilizing Acetogenic Bacteria

Xm : Acetoclastic Methanogenic Bacteria

Xh : Hydrogen Utilizing Methanogenic Bacteria

Figure 2-1 : Microbial ecology of the anaerobic digestion process.

rates. It has been shown that the generation rates of volatile organic acids are influenced by the partial pressure of hydrogen in the gas phase (Iannotti *et al.*, 1973; Kaspar and Whurmann, 1978). When the process is "healthy", the hydrogen utilizers convert most of the hydrogen produced in the acidogenesis step to methane and the concentration of hydrogen in the gas phase is between 1.0 and 10.0 ppm (at atmospheric pressure). However, when the process is imbalanced the hydrogen utilizers are no longer able to convert hydrogen and consequently hydrogen accumulates. Accumulation of hydrogen leads to propionate build up. To quantify this behavior, Mosey (1983) proposed that the distribution of volatile acids produced as acetic, propionic and butyric acids is regulated by the ratio of the concentrations of the reduced to the oxidized forms of the coenzyme nicotinamide adenine dinucleotide ([NADH]/[NAD^+]). This ratio is in turn related to the partial pressure of hydrogen in the gas phase and the pH.

The model presented here is a modification of an earlier modeling effort (Pullammanappallil *et al.*, 1991) and like its predecessor is largely based on the work of Mosey (1983). The regulatory mechanism for the formation of volatile organic acids proposed by Mosey (1983) is integrated with physico-chemical relationships for the liquid and gas phases to predict gas production rates and composition of the gas phase. However, whereas Mosey (1983) assumes a pseudo-steady state for pyruvate and acetylcoenzyme A (acetyl-CoA), which are intermediates during the metabolic conversion of glucose to volatile organic acids, our model does not. Relaxing this

assumption delays accumulation of volatile organic acids in response to an increase in the feed load and as a result the model fits experimental data better.

Costello *et al.* (1991a; 1991b) presented a very similar modeling approach. They also coupled Mosey's distribution of volatile organic acids with equilibrium and mass transfer relationships. However, they did not account for the accumulation of acetyl-CoA and pyruvate while they considered lactic acid as an intermediate.

Another difference between the Costello *et al.* model and the model presented here has to do with the inhibition of the different bacterial populations. Costello *et al.* (1991a) assumed pH inhibition for all the major bacterial populations and in addition hydrogen inhibition for acidogenic, acetogenic and lactic acid bacteria. In our model, the acidogenic, acetogenic and acetoclastic methane bacteria are only inhibited by hydrogen and the hydrogen-utilizing bacteria are pH inhibited. These inhibitions are in better agreement with experimental data (Zehnder, 1977; Chapter 5 this dissertation).

In what follows the model equations are first derived. Parameter determination is subsequently discussed. Next, the experimental set-up, materials and methods used for model verification are presented. Finally, the model is verified by comparing it against experimental data. It is shown that incorporating pyruvate accumulation significantly improves the model performance. However, further improvement by incorporation of acetyl Co-A accumulation is negligible.

Model Development

Development of the process model has been divided into three major sections. The first section details the stoichiometry of the key conversion steps, stoichiometry of the bacterial synthesis steps and the biological rate equations for the bacterial populations. In the second section, physico-chemical relationships for the liquid phase are developed. The third section describes the dynamics of the gas phase.

Stoichiometry and Biological Rate Equations

Bacteria are unable to take up particulate organic material. By the action of hydrolytic enzymes particulates are broken down to soluble intermediates. In this work, glucose is used as a representative molecule to simulate these soluble organic substrates. An empirical formula of $C_5H_9O_3N$ is assumed for all bacterial populations (Mosey, 1983). Bauchop and Elsden (1960) suggest that one mole of ATP provides sufficient energy for the formation of 10 grams of biomass. However, experimental data obtained from the digester used in this study showed that this value substantially overpredicts the amount of biomass produced and that a value of 4.0 grams might be more appropriate. This empirical value (Y_B) is used to determine biomass yield coefficients from the stoichiometric relationships. Following Mosey (1983) all other yield coefficients where determined from the stoichiometry of the conversion reactions.

Acidogenesis step. The acidogenic bacteria convert glucose to primarily acetic, propionic and butyric acids according to the following reactions (Mosey, 1983)

$$C_6H_{12}O_6 + 2H_2O \rightarrow \underset{\text{(acetic)}}{2CH_3COOH} + 2CO_2 + 4H_2 + 4ATP \qquad (2\text{-}1)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow \underset{\text{(propionic)}}{2CH_3CH_2COOH} + 2H_2O + 2ATP \qquad (2\text{-}2)$$

$$C_6H_{12}O_6 \rightarrow \underset{\text{(butyric)}}{CH_3CH_2CH_2COOH} + 2CO_2 + 2H_2 + 2ATP \qquad (2\text{-}3)$$

Under normal conditions most of the glucose is converted to acetic acid. Hydrogen is also produced. This hydrogen is converted to methane by the hydrogen utilizing methane bacteria. However, if for any reason the hydrogen utilizing bacteria are inhibited then hydrogen accumulates. High hydrogen concentrations would favor the formation of propionic acid as can be seen from the above stoichiometric relationship. The butyric acid formation reaction is also favored because this reaction produces less hydrogen than the acetic acid reaction. Hence, an imbalance in the process results in accumulation of propionic and butyric acids.

To quantify this accumulation, it is necessary to look at the metabolic pathway of the acidogenic bacteria. A simplified diagram of this pathway is shown in Figure 2-2. It can be seen that the regulation of the formation of the volatile organic acids is determined by the relative availabilities of the reduced (NADH) and the oxidized (NAD^+) forms of the intracellular coenzyme nicotinamide adenine dinucleotide. High NADH concentrations would favor formation of propionic acid and butyric acid. The NADH half reaction (Figure 2-2) shows that high H^+ concentrations would favor the formation of NADH. An increase in the partial pressure of

hydrogen in the gas phase would result in an increase in the concentration of H^+. To formulate a relationship between the ratio [NADH]/[NAD^+], pH and partial pressure of hydrogen, Mosey (1983) equated the redox potential of the half reaction

$$NAD^+ + H^+ + 2e^- \leftrightharpoons NADH$$

to that of

$$2H^+ + 2e^- \leftrightharpoons H_2$$

and made the following assumptions:

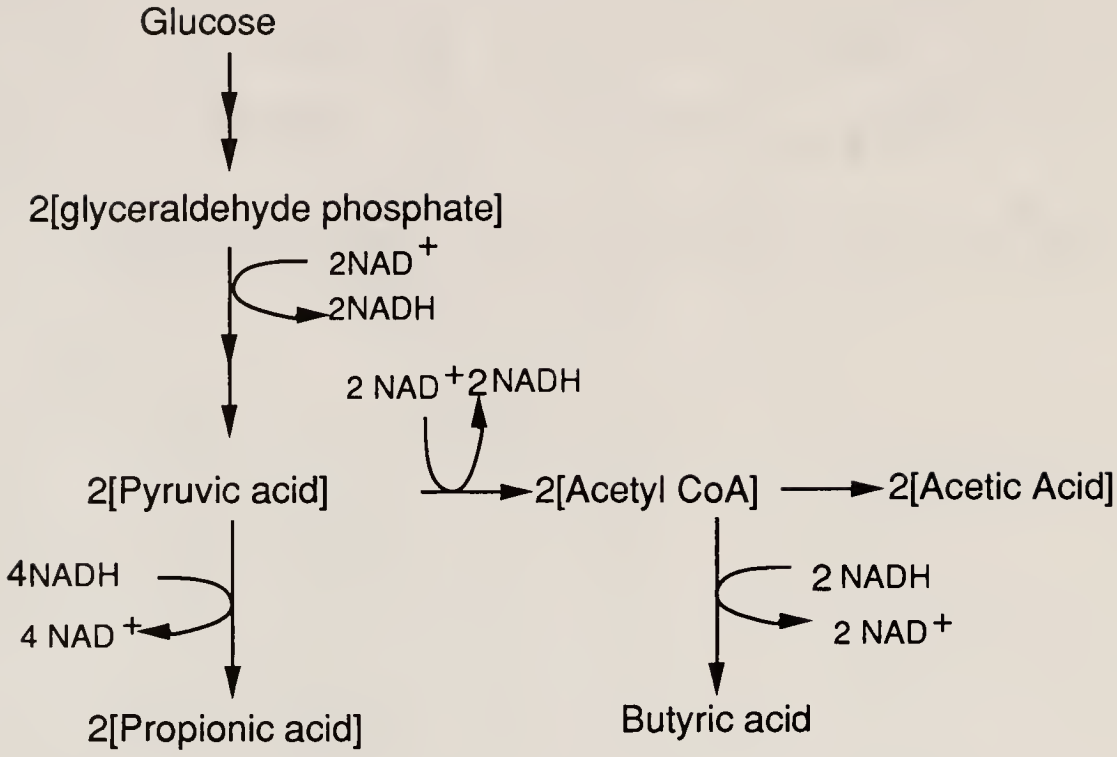
a) gaseous hydrogen diffuses both freely and rapidly into and out of the bacterial cells, so that the concentration of hydrogen inside the bacteria is proportional to the partial pressure of hydrogen in the digester gas,

b) redox potential of the bacteria is equal to the redox potential of the growth medium. These result in the following expression:

$$\frac{[NADH]}{[NAD^+]} = 10^{\left(\log p_{H_2} + pH + \frac{E^\circ}{29.55}\right)}$$

where E° is the standard redox potential of the NADH/NAD^+ couple, i.e. -113 mV.

The conversion of glucose to pyruvate is also affected by the [NADH]/[NAD^+] ratio (Figure 2-2). High concentrations of NAD^+ would maximize this conversion rate. Following Mosey (1983), it is assumed that the uptake of glucose for catabolism follows Monod kinetics and is proportional to the NAD^+ fraction (which is equivalent to inhibition by the [NADH]/[NAD^+] ratio). The rate of utilization of glucose for catabolism is then given by



$$NADH \rightleftharpoons NAD^+ + 2e^- + H^+$$

Figure 2-2: A simplified diagram of the metabolic pathway inside the acidogenic bacteria.

$$r'_G = \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)} \frac{k_G\,[G]}{K_{s,G} + [G]}\,[Xa] \qquad (2\text{-}4)$$

where k_G is the maximum rate constant of the acidogens (mMol/mg acidogen/day) and $K_{s,G}$ the Monod half-velocity constant (mMol/L).

By assuming that there is no accumulation of pyruvate or acetyl-CoA, that the converted pyruvic acid is split between propionic acid and acetyl-CoA according to the NAD^+ fraction and that the converted acetyl-CoA is also split according to the NAD^+ fraction, the rate equations for the formation of acetic, propionic and butyric acids can be formulated (Mosey, 1983):

$$\frac{d[Propionic]}{dt} = \frac{2r'_G}{\left(1 + \frac{[NAD^+]}{[NADH]}\right)} \qquad (2\text{-}5)$$

$$\frac{d[Butyric]}{dt} = \frac{r'_G}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)\left(1 + \frac{[NAD^+]}{[NADH]}\right)} \qquad (2\text{-}6)$$

$$\frac{d[Acetic]}{dt} = \frac{2r'_G}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)^2} \qquad (2\text{-}7)$$

However, experimental data obtained by doubling the glucose concentration in the feed showed that the build up of volatile organic acids started at a considerably lower rate than the above expressions would predict. This indicates that accumulation of at least one intermediate should be considered and leads us to investigate relaxing the pseudo-steady state assumptions for pyruvate and acetyl-CoA.

To account for pyruvate and acetyl-CoA accumulation, it is necessary to devise expressions for the rate of utilization of pyruvate and acetyl-CoA. These expressions are assumed to be of the Michaelis-Menten form (Bailey and Ollis, 1985). It is also assumed that the concentration of enzymes that convert pyruvate to propionate and acetyl-CoA to butyrate and acetate are proportional to the concentration of the acidogenic biomass. Hence, the utilization rate expressions are

$$r'_{PYR} = \frac{\alpha\ [PYR]}{K_{s,PYR} + [PYR]}\ [Xa] \qquad (2\text{-}8)$$

$$r'_{CoA} = \frac{\beta\ [CoA]}{K_{s,CoA} + [CoA]}\ [Xa] \qquad (2\text{-}9)$$

The net rates of formation for pyruvate and acetyl-CoA are

$$\frac{d[Pyruvate]}{dt} = 2r'_G - r'_{PYR}$$

$$\frac{d[CoA]}{dt} = \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)} r'_{PYR} - r'_{CoA}$$

Based on the above assumptions and rate expressions the rate of formation of volatile organic acids then become

$$\frac{d[Propionic]}{dt} = \frac{1}{\left(1 + \frac{[NAD^+]}{[NADH]}\right)} r'_{PYR} \qquad (2\text{-}10)$$

$$\frac{d[Butyric]}{dt} = \frac{1}{2} \frac{1}{\left(1 + \frac{[NAD^+]}{[NADH]}\right)} r'_{CoA} \qquad (2\text{-}11)$$

$$\frac{d[Acetic]}{dt} = \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)} r'_{CoA} \qquad (2\text{-}12)$$

As will be shown later, by considering only accumulation of pyruvate and assuming pseudo-steady state for acetyl-CoA the model predictions do not change significantly. In that case

$$\frac{d[CoA]}{dt} = 0$$

i.e.,

$$r'_{CoA} = \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)} r'_{PYR}$$

The net rates of formation of the volatile organic acids are obtained by replacing r'_{CoA} from the above expression in equations (7) through (9).

The stoichiometry for the synthesis of acidogenic biomass (Mosey 1983) is

$$5C_6H_{12}O_6 + 6NH_3 \rightarrow 6C_5H_9O_3N + 12H_2O \qquad (2\text{-}13)$$

The overall synthesis rate is due to synthesis in the acetic acid formation step, propionic acid formation step and butyric acid formation step. The net rate of utilization of glucose by the acidogenic biomass would then be the sum of the rate of glucose utilized for catabolic activity and the utilization rate for synthesis of biomass.

$$\frac{d[Glucose]}{dt} = r'_G + \frac{1}{Y^{Ad,s}_{Xa/G}} \left(Y^{Ad}_{Xa/A} \frac{d[Acetic]}{dt} + Y^{Ad}_{Xa/P} \frac{d[Propionic]}{dt} + Y^{Ad}_{Xa/B} \frac{d[Butyric]}{dt}\right)$$

The parameters Y are yield coefficients which are determined from stoichiometry along with the empirical yield of 4.0 grams of biomass per mole of ATP. The notation is explained in Appendix A.

Acetogenesis step. In this step longer acids, namely propionic and butyric acids, are converted to acetic acid, hydrogen and carbon dioxide. The propionate-utilizing acetogens consume propionate and the stoichiometry for this conversion process is (Mosey, 1983)

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 + 1ATP \quad (2\text{-}14)$$

The stoichiometry for the butyrate utilizing organisms is (Mosey, 1983)

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 + 2ATP \quad (2\text{-}15)$$

From the above stoichiometric equations, it can be seen that the conversion processes are inhibited by high hydrogen concentrations.

The specific growth rate expressions for the acetogens are of the Monod form with terms incorporated for inhibition by hydrogen (Mosey, 1983)

$$\mu_{Xp} = \frac{\mu_{max,Xp}\,[\text{Propionic}]}{(K_{s,P} + [\text{Propionic}])\,(1 + KIH_{Xp}p_{H_2})} \quad (2\text{-}16)$$

$$\mu_{Xb} = \frac{\mu_{max,Xb}\,[\text{Butyric}]}{(K_{s,B} + [\text{Butyric}])\,(1 + KIH_{Xb}p_{H_2})} \quad (2\text{-}17)$$

The stoichiometry for synthesis of the propionate-utilizing and butyrate-utilizing biomass (Mosey, 1983) is

$$3CH_3CH_2COOH + CO_2 + 2NH_3 \rightarrow 2C_5H_9O_3N + 2H_2O + H_2 \quad (2\text{-}18)$$

$$CH_3CH_2CH_2COOH + CO_2 + NH_3 \rightarrow C_5H_9O_3N + H_2O \quad (2\text{-}19)$$

respectively. The rate of utilization of propionate and butyrate can be expressed as a product of the growth rate of the acetogens and a yield coefficient (mg of acetogens/ mMol of propionate (or butyrate) consumed) which is determined from the above stoichiometry.

Acetoclastic methanogenesis step. Almost 70 percent of the methane produced is due to this step. The acetoclastic methanogens convert the acetic acid produced in the previous steps to methane and carbon dioxide. The stoichiometry for this step (Mosey, 1983) is

$$CH_3COOH \rightarrow CH_4 + CO_2 + 0.25ATP \qquad (2\text{-}20)$$

The model presented by Smith *et al.* (1988) assumes that the acetoclastic methanogens are inhibited by acetic acid, the substrate and consequently they incorporate a inhibition factor for acetic acid inhibition in the growth rate expression. Several investigators have studied volatile organic acid toxicity in anaerobic digestion (Buswell, 1947; Schlenz, 1947; McCarty and Mckinney, 1961; Buswell and Morgan, 1962; Kugelman and Chin, 1971; Jarrell *et al.*, 1987; Barredo and Evison, 1991). Buswell (1947) and Schlenz (1947) reported that volatile organic acids are toxic to methane bacteria at concentrations of about 2000 mg/L. McCarty and McKinney (1962) investigated the toxicity of acetic acid to methane bacteria and found that toxicity sets in at concentrations between 2000 and 4000 mg/L. However, they concluded that the toxicity was caused by the sodium cation (which was added at increased amounts as part of the buffer). Following this, Buswell and Morgan (1962) reported that propionic rather than acetic acid was toxic to methane bacteria.

Subsequently McCarty and co-workers reported that volatile organic acids did not adversely affect methanogenesis but high concentrations of propionate (6000 mg/L) inhibited the acidogenic bacteria (Kugelman and Chin, 1971). Jarrell *et al.* (1987) reported that propionate and butyrate can be inhibitory at concentrations above 60 g/L. However, Barredo and Evison (1991) found that concentrations of propionate as low as 1480 mg/L can cause inhibition of the methanogens. Hence, the debate over toxicity of the volatile organic acids still continues. But studies that were done in our laboratory (detailed in Chapter 5) showed that the methanogens were not inhibited even at propionate concentrations of 3500 mg/L. Therefore, inhibition of acetoclastic methanogens by volatile organic acids is omitted in the present model development. It is proposed instead, that the kinetics of these organisms are regulated by hydrogen. It has been shown that most species of methanogens share the common ability to form methane via reduction of carbon dioxide with hydrogen (Oremland, 1988). When hydrogen accumulates in the digester, due to reasons other than inhibition of hydrogen utilizers, it becomes thermodynamically favorable for hydrogen to be utilized rather than acetate. This is evident when the energy yields by the two substrates acetate and hydrogen are compared. The energy yield by acetate is -28 kJ/mol whereas by hydrogen it is -139 kJ/mol. Incorporating inhibition by hydrogen, the specific growth rate of the acetoclastic methanogens can be written as follows

$$\mu_{Xm} = \frac{\mu_{max,Xm}\,[Acetic]}{([Acetic] + K_{s,A})\,(1 + KIH_{Xm}p_{H_2})} \qquad (2\text{-}21)$$

The stoichiometry for the synthesis of acetoclastic methanogenic biomass (Mosey, 1983) is

$$5CH_3COOH + 2NH_3 \rightarrow 2C_5H_9O_3N + 4H_2O \qquad (2\text{-}22)$$

This is used to determine the amount of acetic acid needed for synthesis of methanogenic biomass.

Methanogenesis from hydrogen. The hydrogen-utilizing methanogens reduce carbon dioxide with hydrogen according to the following stoichiometry (Mosey, 1983):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O + 1ATP \qquad (2\text{-}23)$$

The hydrogen utilizers are assumed to follow simple Monod kinetics, regulated by the pH of the fermentation broth. Zehnder and Wuhrmann (1977) has shown that maximum growth occurs at a pH of around 7.1 and that the maximum specific growth rate drops by half at pH below 6.6 and above 7.6. The functional form of pH inhibition taken from Bailey and Ollis (1985)

$$\mu_{max,Xh} = \frac{K}{\left(1 + \frac{[H^+]}{b} + \frac{c}{[H^+]}\right)}$$

fits Zehnder and Wuhrmann's data well with values K = 31.93, b = 1.273×10^{-8} and c = 7.58×10^{-7}. Hydrogen is the limiting substrate for this microbial conversion step. Since hydrogen is sparingly soluble in water, the concentration of hydrogen in the

fermentation broth is assumed to be proportional to the partial pressure of hydrogen in the gas phase. The specific growth rate then is

$$\mu_{Xh} = \frac{\mu_{max,Xh}\ p_{H_2}}{(K_{s,Xh} + p_{H_2})} \qquad (2-24)$$

The stoichiometry for synthesis of hydrogen utilizers is

$$5CO_2 + 10H_2 + NH_3 \rightarrow C_5H_9O_3N + 7H_2O \qquad (2\text{-}25)$$

The above equation is used to determine the amount of hydrogen and carbon dioxide needed for the formation of biomass and the values are incorporated into the carbon dioxide and hydrogen utilization rates.

The kinetic expressions for substrate utilization and biomass growth based on the stoichiometry discussed above are presented in Appendix A.

The Physico-Chemical Relationships

Relationships based on the physico-chemical properties of the various ionic reactions are developed so as to be able to calculate ion concentrations in the digester fermentation broth. The ionic species commonly found are OH^-, H^+, CO_3^{2-}, HCO_3^-,A^-,P^-,B^-, other cations (C_{ions}) and other anions (A_{ions}). A^-,P^- and B^- are the ionized forms of acetic, propionic and butyric acids. To specify completely a physico-chemical system, the overall charge balance equation is required along with a mass balance equation and an equilibrium relationship for each component species. It is assumed that $(CO_2)_{dissolved}$-HCO_3^- is the only buffering system present.

Dissociation of acetic, propionic and butyric acids can be written as follows

$$AH \leftrightharpoons A^- + H^+ \quad (K_A\text{:equilibrium constant})$$

$$PH \leftrightharpoons P^- + H^+ \quad (K_P\text{:equilibrium constant})$$

$$BH \leftrightharpoons B^- + H^+ \quad (K_B\text{:equilibrium constant})$$

The total concentrations of each volatile organic acid are the sum of the ionized form and the unionized form. Hence, the total concentrations of acetic, propionic and butyric acids are

$$[A] = [AH] + [A^-]$$

$$[P] = [PH] + [P^-]$$

$$[B] = [BH] + [B^-]$$

respectively. Assuming equilibrium, the concentration of the ionized acids can be expressed as

$$[A^-] = \frac{K_A [A]}{(K_A + [H^+])} \qquad (2\text{-}26)$$

$$[P^-] = \frac{K_P [P]}{(K_P + [H^+])} \qquad (2\text{-}27)$$

$$[B^-] = \frac{K_B [B]}{(K_B + [H^+])} \qquad (2\text{-}28)$$

The dissolution of carbon dioxide gives

$$(CO_2)_D + H_2O \rightleftharpoons HCO_3^- + H^+ \quad (K_{bc}\text{:equilibrium constant})$$

where $(CO_2)_D$ represents carbon dioxide dissolved in the liquid. Since anaerobic digesters are usually operated at pH below 8, the concentration CO_3^{-2} is neglected. The concentration of the total inorganic carbon then is

$$[TC] = [(CO_2)_D] + [HCO_3^-]$$

From the equilibrium relationship, the concentration of HCO_3^- is

$$[HCO_3^-] = \frac{K_{bc}\,[TC]}{(K_{bc} + [H^+])} \qquad (17)$$

For pH < 8 the concentration of OH^- can also be neglected and the overall charge balance is

$$[Z] + [H^+] = [A^-] + [P^-] + [B^-] + [HCO_3^-]$$

where

$$[Z] = \text{net cations} = [\text{Other cations}] - [\text{Other anions}]$$

Substituting for the ionized forms of the volatile organic acids and HCO_3^- from equations (14), (15), (16) and (17) into the above charge balance yields

$$[Z] + [H^+] = \frac{K_A\,[A]}{(K_A + [H^+])} + \frac{K_P\,[P]}{(K_P + [H^+])} + \frac{K_B\,[B]}{(K_B + [H^+])} + \frac{K_{bc}\,[TC]}{(K_{bc} + [H^+])}$$

If [Z] and [TC] are known, the above equation is a fifth order polynomial in $[H^+]$ and it can be solved numerically. However, the values of the dissociation constants for the volatile organic acids are close to each other. For example at 35°C and ionic

strength of 0.02, $K_A = 1.76 \times 10^{-5}$, $K_B = 1.54 \times 10^{-5}$ and $K_P = 1.34 \times 10^{-5}$. Hence, it is reasonable to approximate the dissociation constants of propionic and butyric acids by that of acetic acid and the equivalent dissociation constant is denoted by K_e. This approximation reduces the above equation to a cubic, which can then be solved analytically. If very low values of pH (< 5) are not of interest, then the polynomial becomes an easy to handle quadratic by neglecting the $[H^+]$ term on the left. In this study $[H^+]$ is not neglected and the cubic equation is solved analytically.

[Z] and [TC] can be obtained by setting up respective mass balances. Net cations would be in the form of $[NH_4^+]$ and $[Na^+]$ since $NaHCO_3$ and NH_4HPO_4 are the only buffers added to the feed. Following Smith *et al.* (1988), the net cations are assumed to be consumed at a rate proportional to the growth of biomass from all species. The mass balance for [Z] for a continuously stirred anaerobic digester is given in Appendix A

A mass balance for [TC] yields

$$\frac{d[TC]}{dt} = D\left([(CO_2)_D]_o + [HCO_3^-]_o - [TC]\right) + r_{CO_2} + T_g$$

where $[(CO_2)_D]_o$ = dissolved carbon dioxide concentration in the feed

$[HCO_3^-]_o$ = bicarbonate ion concentration in the feed

r_{CO2} = net rate of carbon dioxide generation from the biological reactions

T_g = carbon dioxide transfer rate from gas phase to liquid

The rate of generation (or consumption) of carbon dioxide is proportional to the growth rate of the bacteria that are generating (or consuming) it and an expression is given in Appendix A. The carbon dioxide transfer rate is given by

$$T_g = KL_a (K_{HL} p_{CO_2} - [(CO_2)_D])$$

where KL_a is the mass transfer coefficient and K_{HL} is the Henry's Law constant.

Gas Phase Equations

The gas phase is assumed to be completely mixed and always at a total pressure of one atmosphere. Moisture content of the gas is neglected so that

$$p_{CO_2} + p_{H_2} + p_{CH_4} = P_T = 1 \text{ atm}$$

Methane and hydrogen are assumed to be insoluble in the fermentation broth and all three gases follow ideal gas behavior. Then the mass balances for the gas phase can be written in terms of the partial pressures

$$\frac{dp_{CO_2}}{dt} = \frac{P_T Q_{CO_2}}{V_g} - \frac{p_{CO_2} Q_{gas}}{V_g}$$

$$\frac{dp_{H_2}}{dt} = \frac{P_T Q_{H_2}}{V_g} - \frac{p_{H_2} Q_{gas}}{V_g}$$

$$\frac{dp_{CH_4}}{dt} = \frac{P_T Q_{CH_4}}{V_g} - \frac{p_{CH_4} Q_{gas}}{V_g}$$

Q_{CO_2}, Q_{H_2}, Q_{CH_4} are volumetric rates of production in the liquid phase for CO_2, H_2 and CH_4 respectively, $Q_{gas} (= Q_{CO_2} + Q_{H_2} + Q_{CH_4})$ the rate of gas leaving the digester and V_g the constant volume of the gas phase. Now

$$Q_{CO_2} = -T_g \hat{V}_{id} V$$

$$Q_{H_2} = r_{H_2} \hat{V}_{id} V$$

$$Q_{CH_4} = r_{CH_4} \hat{V}_{id} V$$

where

V = volume of the liquid phase

r_{H2} = net rate of formation of hydrogen from the biological reactions (see Appendix A)

r_{CH4} = rate of formation of methane from the methanogenesis steps (see Appendix A)

$\hat{V}_{id}$ = volume of one mole of gas, in liters, assuming ideal behavior, at 35 °C

The complete model for a glucose fed anaerobic digester is summarized in Appendix A.

Determination of Model Parameters

All the yield coefficients except Y_B and Y_Z are calculated from the stoichiometric relationships presented in the above section. Y_Z was estimated such that model pH predictions fit experimental pH data. The value of Y_B was estimated such that the total biomass concentration calculated by the model fits experimental data. Table 2-1 lists the values and the stoichiometric equation or source, of all the yield coefficients used in the model. The notation is explained in Appendix A. The physico-chemical parameters were obtained from the literature and is listed in Table 2-2.

The kinetic parameters, maximum growth rate (μ_{max}) and Monod half-velocity constant (K_s), for all bacterial species except for that of the acetoclastic methanogens are obtained from the literature. However, for the acetoclastic methanogens a range of values were available in the literature. Several researchers have calculated the values for the kinetic parameters $\mu_{max,Xm}$ and $K_{s,A}$ (Lawrence and McCarty, 1969; Smith and Mah, 1980; Zehnder *et al.*, 1980; Gujer and Zehnder, 1983; Smith *et al.*, 1988). The values reported in these papers vary significantly. The value of $\mu_{max,Xm}$ varies from 0.11 d^{-1} to 0.54 d^{-1} and the value of $K_{s,A}$ ranges from 0.44 mMol/l to 14.53 mMol/l. Hence, it was appropriate to estimate the values of these parameters

Table 2-1
Yield Coefficients (expressed in Mol/Mol)

Yield	Value	Source	Yield	Value	Source
Y_B	0.031	Expt.	$Y^{At}_{H_2/B}$	2	2-15
$Y^{Ad}_{Xa/A}$	0.062	2-1	$Y^{At,s}_{Xp/H_2}$	2	2-18
$Y^{Ad}_{Xa/P}$	0.031	2-2	$Y^{At,s}_{Xb/B}$	1	2-19
$Y^{Ad}_{Xa/B}$	0.062	2-3	$Y^{At}_{CO_2/P}$	1	2-14
$Y^{Ad}_{CO_2/A}$	1	2-1	$Y^{At,s}_{CO_2/Xp}$	0.5	2-18
$Y^{Ad}_{CO_2/B}$	2	2-3	$Y^{At,s}_{CO_2/Xb}$	1	2-19
$Y^{Ad}_{H_2/A}$	2	2-1	$Y^{M}_{Xm/A}$	0.008	2-20
$Y^{Ad}_{H_2/P}$	1	2-2	$Y^{M,s}_{Xm/A}$	0.4	2-22
$Y^{Ad}_{H_2/B}$	2	2-3	$Y^{M}_{CO_2/A}$	1	2-20
$Y^{Ad,s}_{Xa/G}$	1.2	2-13	$Y^{M}_{CH_4/A}$	1	2-20
$Y^{At}_{A/P}$	1	2-14	$Y^{H}_{CO_2/H_2}$	4	2-23
$Y^{At}_{Xp/P}$	0.031	2-14	Y^{H}_{Xh/H_2}	0.008	2-23
$Y^{At,s}_{Xp/P}$	0.667	2-18	$Y^{H,s}_{CO_2/Xh}$	5	2-25
$Y^{At}_{A/B}$	2	2-15	$Y^{H,s}_{Xh/H_2}$	0.1	2-25
$Y^{At}_{Xb/B}$	0.062	2-15	$Y^{H}_{CH_4/H_2}$	0.25	2-23
$Y^{At}_{H_2/P}$	3	2-14	Y_Z	1.39	Expt.

Table 2-2
Physico-chemical Parameters

Dissociation constant of the dissolved carbon dioxide-bicarbonate system, K_{bc} (Mol/L)	$1 \times 10^{-6.32}$
Equivalent dissociation constant of volatile organic acids, K_e (Mol/L)	1.76×10^{-5}
Henry's Law constant, K_{HL} (Mol/atm/L)	$1 \times 10^{-1.5}$
Overall mass transfer coefficient, KL_a ($days^{-1}$)	100
Gas Constant, $\hat{V}_{id}$ (L/Mol)	25.27

Table 2-3
Kinetic Parameters

Maximum Growth Rates		
Acidogens, k_G (mMol glucose/mg acidogen/day)	1.5	Ghosh and Pohland (1974)
Propionate utilizing acetogens, $\mu_{max,Xp}$ ($days^{-1}$)	0.31	Lawrence and McCarty (1969)
Butyrate utilizing acetogens, $\mu_{max,Xb}$ ($days^{-1}$)	0.61	Ghosh and Klass (1978)
Acetoclsatic methanogens, $\mu_{max,Xm}$ ($days^{-1}$)	0.38	This study
Monod Half-Velocity Constants		
Acidogens, $K_{s,G}$ (mMol/L)	2.21	Ghosh and Pohland (1974)
Propionate utilizing acetogens, $K_{s,P}$ (mMol/L)	0.54	Lawrence and McCarty (1969)
Butyrate utilizing acetogens, $K_{s,B}$ (mMol/L)	0.0813	Lawrence and McCarty (1969)
Acetoclastic methanogens, $K_{s,Xm}$ (mMol/L)	0.54	This study
Hydrogen utilizing methanogens, $K_{s,Xh}$ (atm)	0.014	Shea *et al.* (1968)
Inhibition parameters		
Propionate utilizing acetogens by hydrogen, KIH_{Xp} (atm^{-1})	1548.1	This study
Butyrate utilizing acetogens by hydrogen, KIH_{Xb} (atm^{-1})	3903.1	This study
Acetoclastic methanogens by hydrogen, KIH_{Xm} (atm^{-1})	3547.8	This study
Kinetic parameters for pyruvate utilization		
Maximum rate constant, α (mMol pyruvate/mg acidogen/day)	0.0183	This study
Michaelis constant, $K_{s,PYR}$ (mMol/L)	2.6	This study

from an optimal fit. The estimated values for these parameters were determined to be satisfactory since these were within the ranges mentioned above. It was also necessary to estimate the values for the parameters α, $K_{s,PYR}$, β and $K_{s,CoA}$ since no literature values were available. Table 2-3 lists the values of all the kinetic parameters used in the model. For those parameters whose values were obtained from literature then the reference paper is indicated in the table.

Experimental data obtained by doubling the feed loading rate to a continuously stirred anaerobic digester were used to determine model parameters. The optimal estimates were obtained by minimizing the normalized sum of squared errors between the experimental values and model predictions. Normalization of data was done by dividing acetate concentrations by 100, propionate concentrations by 150, butyrate concentrations by 18 and methane production rate by 1. The optimal estimates were calculated using the Marquadt-Levenberg algorithm (Press *et al.*, 1989). The QuickBASIC 4.5 version of this algorithm presented in Sprott *et al.* (1991) was implemented on a 50 MHz IBM 80486 personal computer to perform the computations.

Materials and Methods

A continuously-fed continuously-stirred (CSTR) 6 L digester was instrumented for automated data acquisition of methane production rate, temperature and pH. The digester was fed by a computer controlled peristaltic pump and temperature

controlled by a PID temperature controller with set-point control provided by the computer. A schematic of the set-up is detailed in Figure 2-3. The computer was equipped with a digital I/O interface board for monitoring a float switch on a U-tube gas meter. The gas vented from the digester was scrubbed of its carbon dioxide content before it reached the gas meter. The methane accumulated in one limb of the U-tube by displacing the liquid. When the liquid in the other limb rose to a certain level, a float switch was tripped, which caused two events to happen simultaneously. A signal was sent to the computer and also the methane from the first limb was exhausted resetting the liquid level. By measuring the time between two successive signals and given that the volume of methane that triggers the signal is known, the methane production rate could be calculated. The digester was operated at a 20 day retention time with a nominal loading rate of 2 g COD L^{-1} d^{-1}. Glucose and feed media were sterilized and kept refrigerated during digester operation. Appendix B lists the components of the feed. Digester effluent was collected from an overflow tube.

Volatile organic acids were measured on an FID gas chromatograph. The samples were prepared by centrifugation followed by sample acidification using 20% phosphoric acid. The samples were injected onto a 2 m long by 2 mm id glass column packed with 80/100 chromosorb 1200 WAW coated with 3% H_3PO_4. A 1 μL volume was injected at an inlet temperature of 180 °C with column temperature ramped from 130 °C to 170 ° over 5 minutes and a detector temperature of 200 °C Nitrogen was used as the carrier gas.

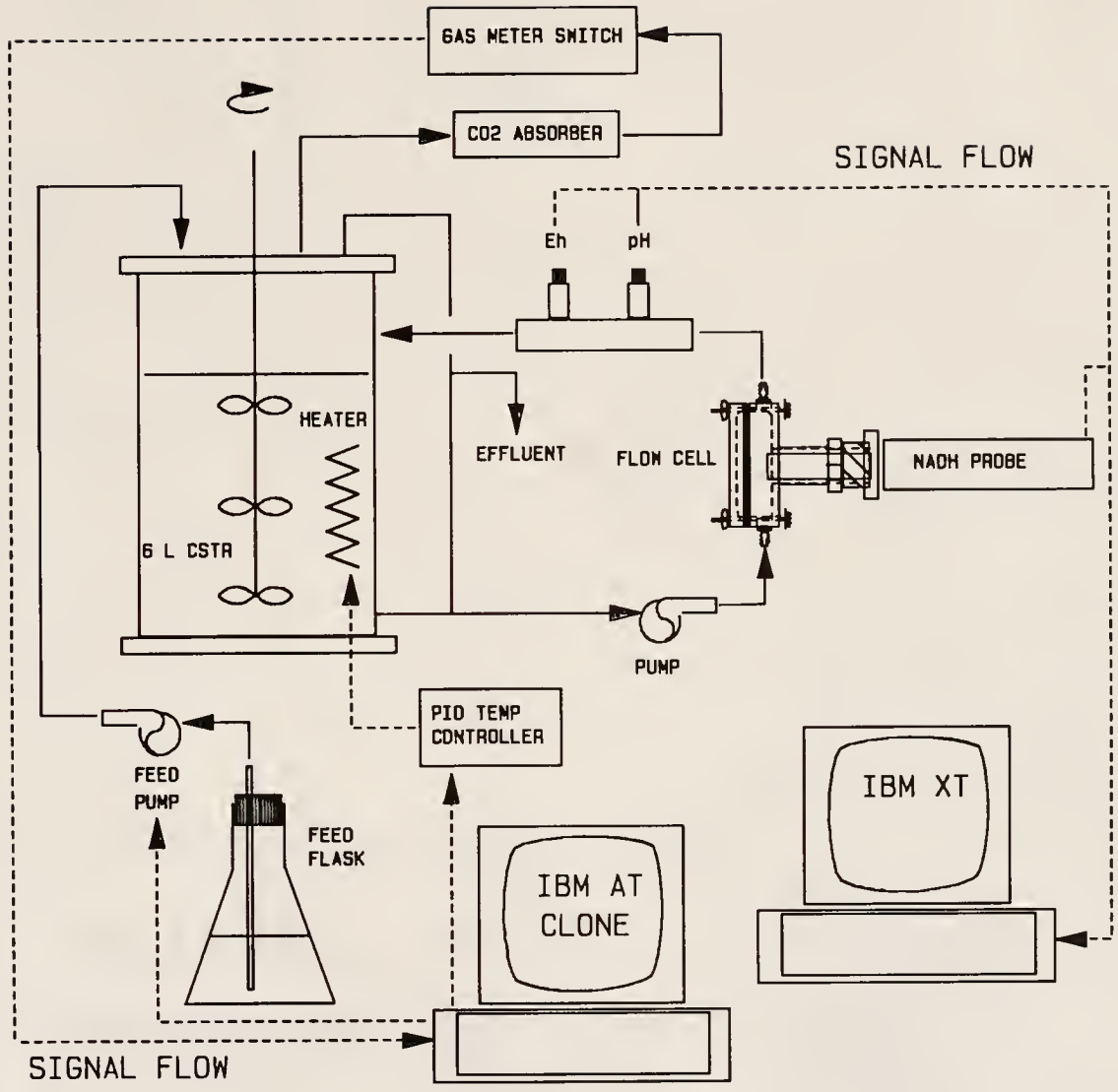


Figure 2-3 : Schematic diagram of the experimental set-up

Model Verification

A dynamic test was first conducted. In this experiment the glucose concentration in the feed was doubled by increasing it from 28.8 g/L to 57.6 g/L. The concentration of the other components of the feed media were not changed. Figures 2-4 through 2-11 compare the experimental results to the optimal model fits. Certain parameters, as mentioned earlier, were estimated by fitting the model to the experimental data.

First, it was assumed that there was no accumulation of pyruvate or acetyl-CoA. The best fit of the model to the experimental acetic acid, propionic acid, butyric acid concentrations as well as the methane production rate is shown in Figures 2-4, 2-5, 2-6 and 2-7 respectively. These figures show that the model response to the feed overload in terms of the accumulation of propionic and butyric acids and rise in methane rate was much faster than the actual result. The acetic acid accumulation rates calculated by the model were comparable to experimental results, with the maximum build up of acetic acid underpredicted. The model predicted the methane rate to increase instantaneously. It also predicted an overshoot. This is contrary to the observations, where the methane rate increased gradually.

Figures 2-8, 2-9, 2-10 and 2-11 show the best fit of the model to experimental acetic acid, propionic acid, butyric acid and methane production rate data, allowing accumulation of pyruvate and acetyl-CoA. The model did reasonably well in

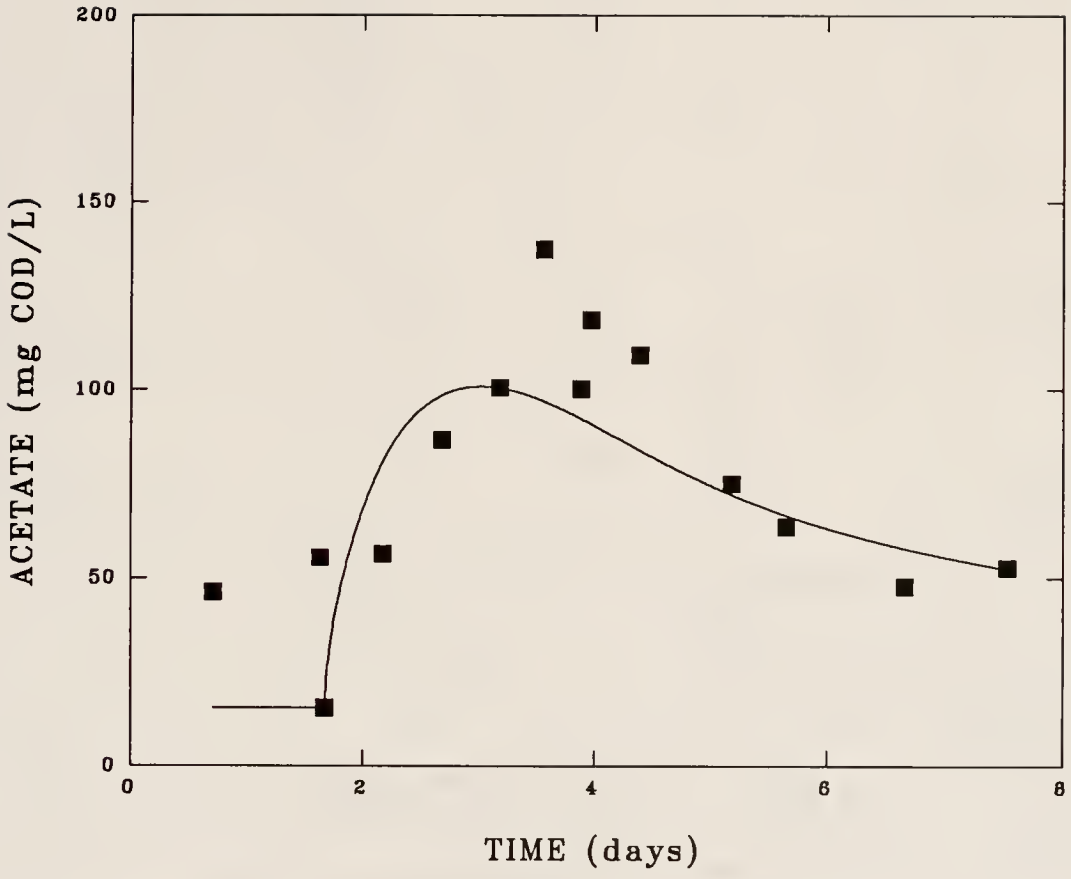


Figure 2-4: Optimal fit of the model assuming no accumulation of pyruvic acid or acetyl-CoA to experimental acetic acid data.

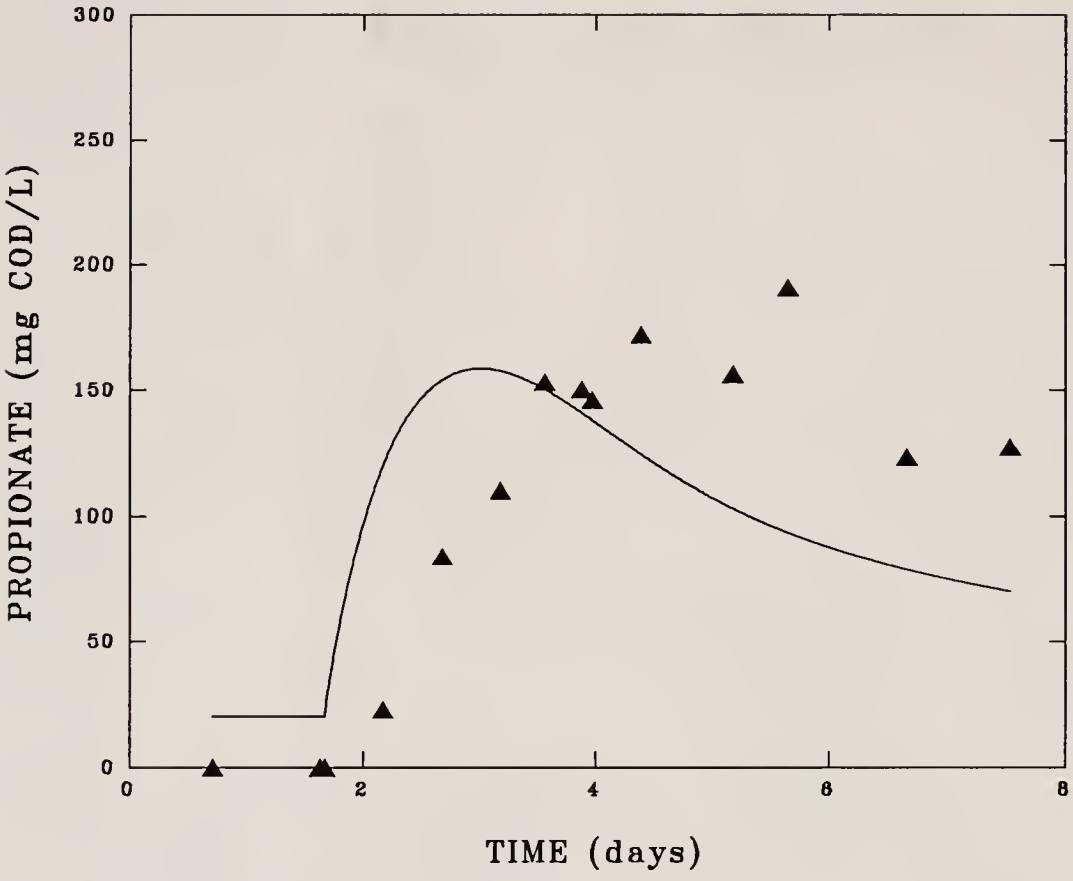


Figure 2-5: Optimal fit of the model assuming no accumulation of pyruvic acid or acetyl-CoA to experimental propionic acid data.

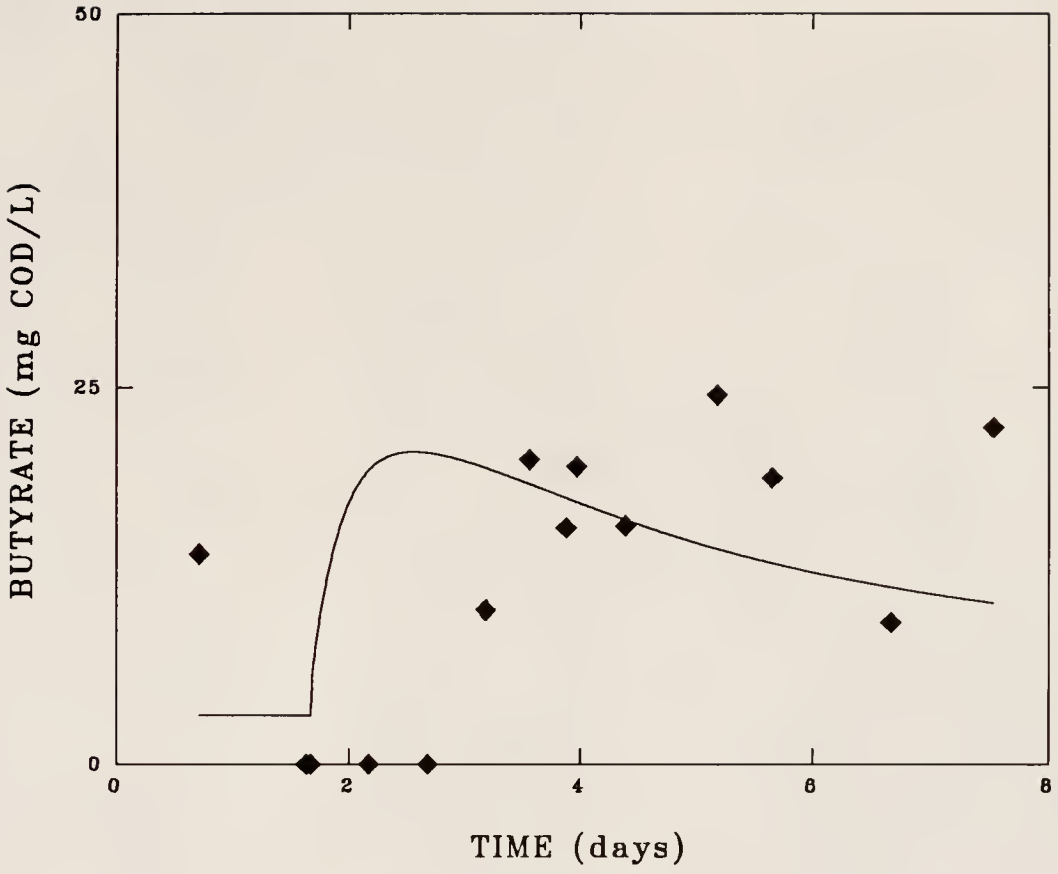


Figure 2-6: Optimal fit of the model assuming no accumulation of pyruvic acid or acetyl-CoA to experimental butyric acid data.

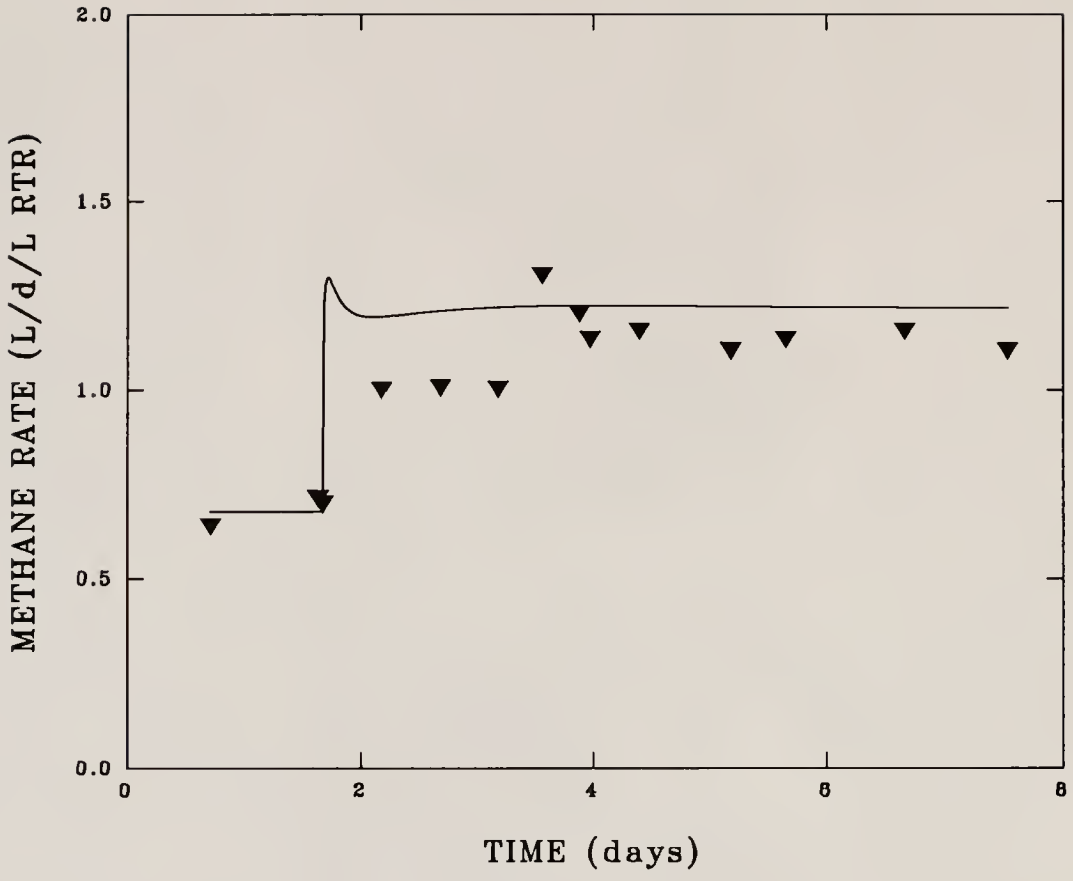


Figure 2-7: Optimal fit of the model assuming no accumulation of pyruvic acid or acetyl-CoA to experimental methane production rate data.

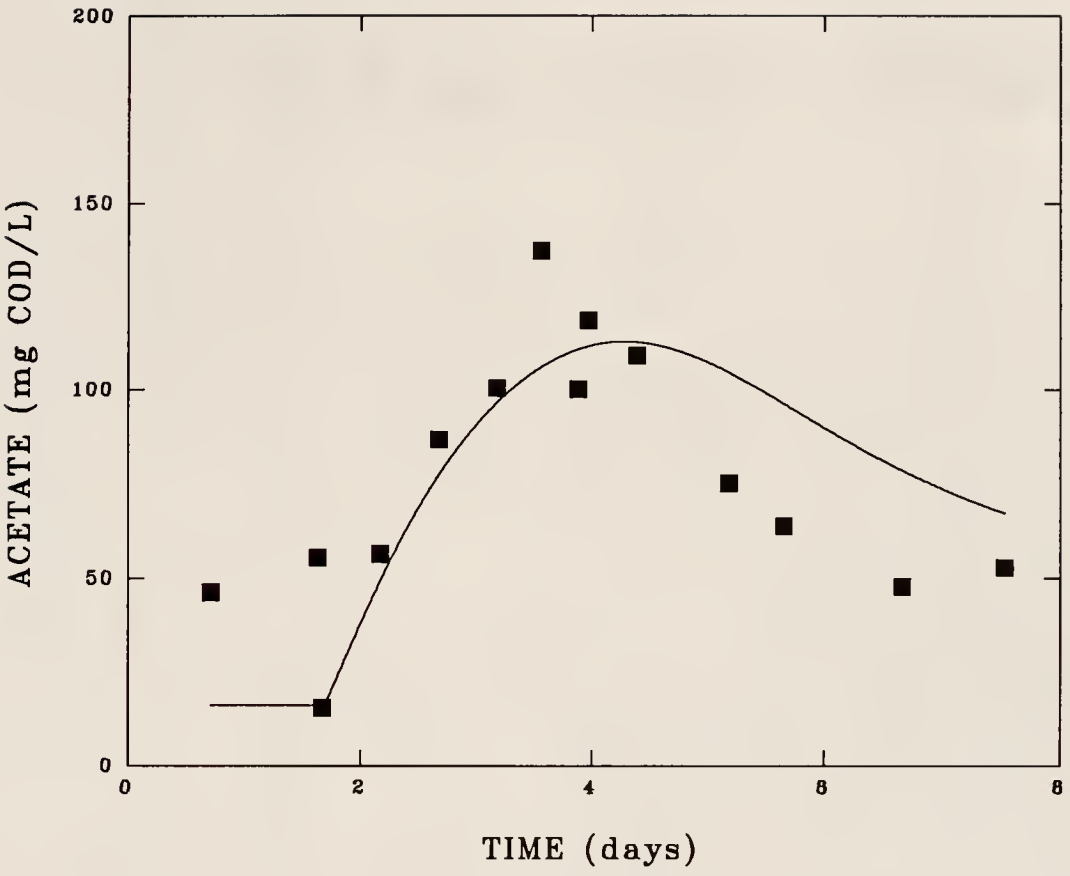


Figure 2-8: Optimal fit of the model assuming accumulation of pyruvic acid (and acetyl-CoA) to experimental acetic acid data.

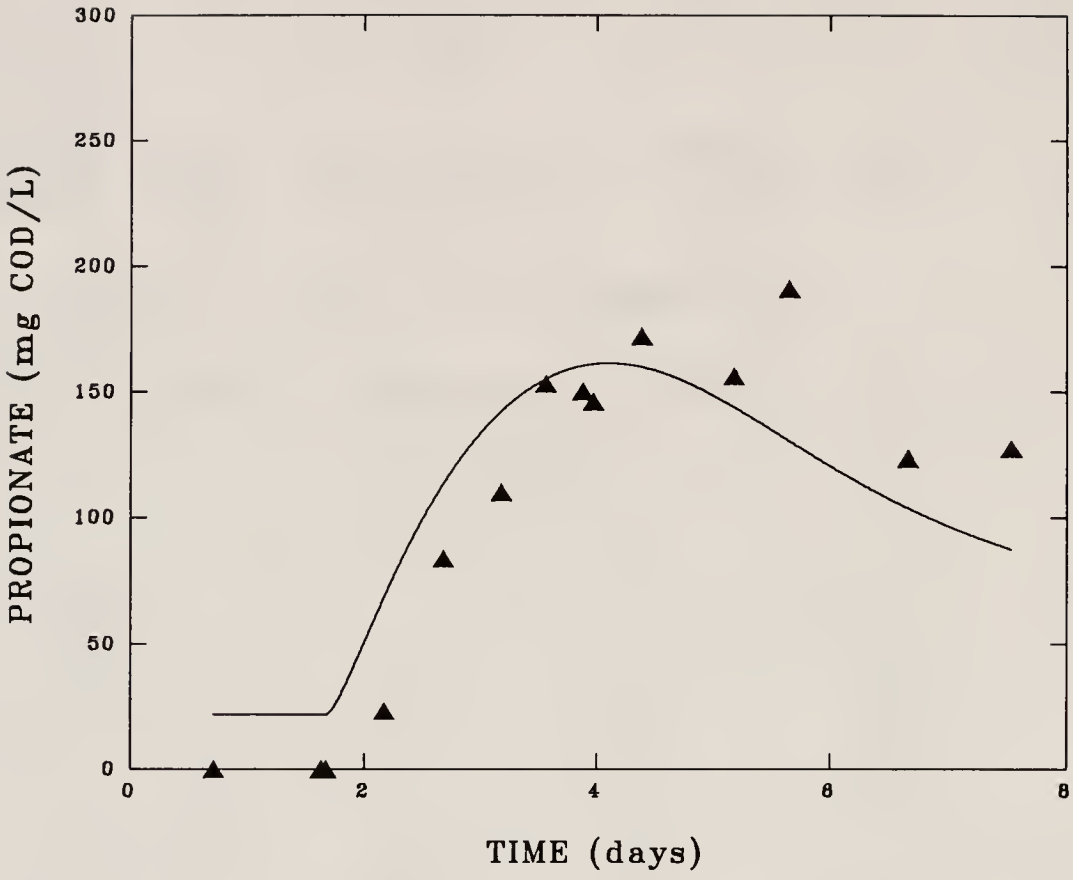


Figure 2-9: Optimal fit of the model assuming accumulation of pyruvic acid (and acetyl-CoA) to experimental propionic acid data.

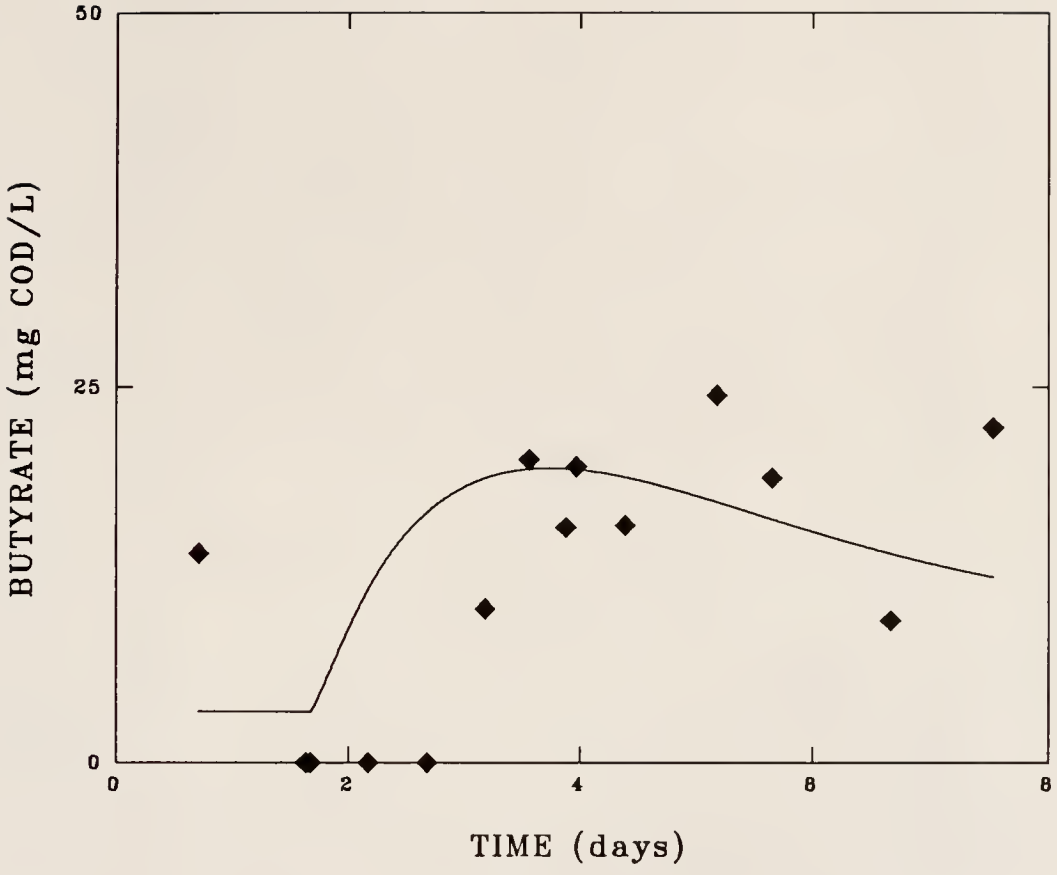


Figure 2-10: Optimal fit of the model assuming accumulation of pyruvic acid (and acetyl-CoA) to experimental butyric acid data.

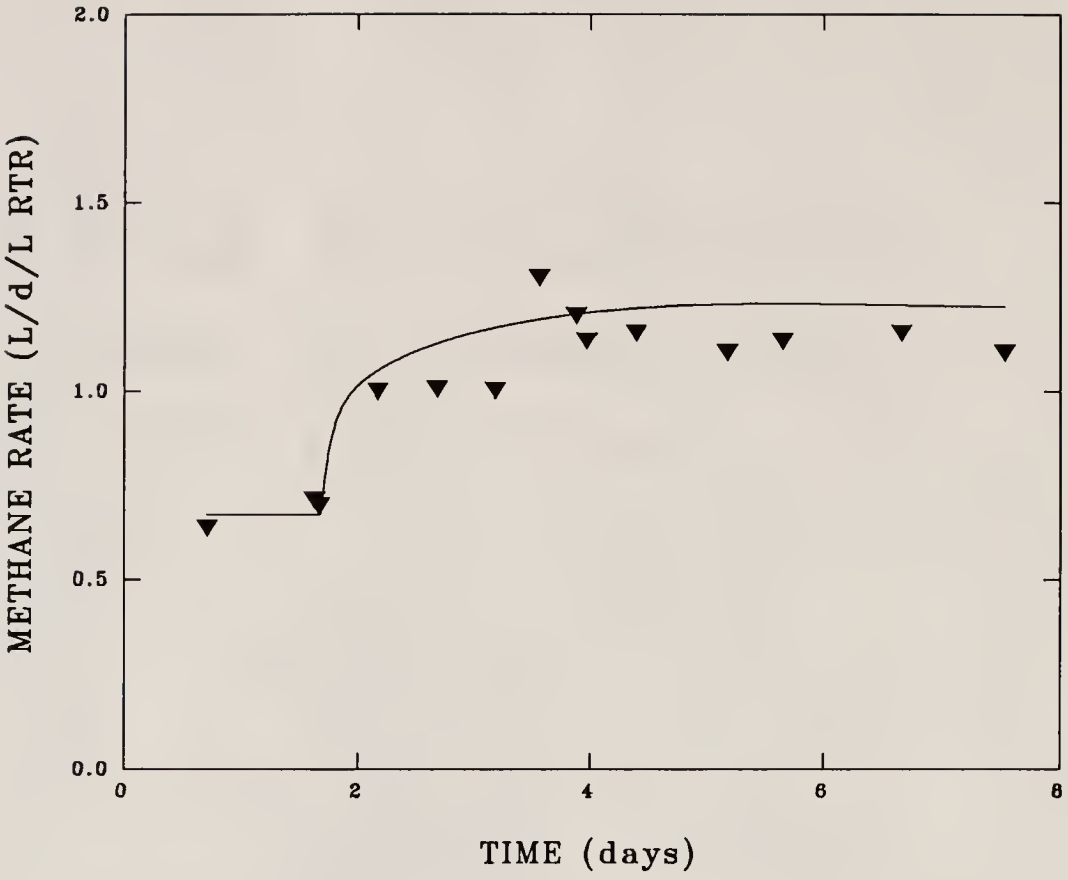


Figure 2-11:Optimal fit of the model assuming accumulation of pyruvic acid (and acetyl-CoA) to experimental methane production rate data.

predicting both the accumulation rate as well as the maximum build up of acetic acid and propionic acid. However, experimental butyrate accumulation (Figure 2-10) occurred later than the model indicates. The calculated methane-rates were in fairly good agreement with the experimental data. The kinetic parameters ß (maximum rate constant) and ($K_{s,CoA}$) (Michaelis constant) for acetyl-CoA accumulation that gave the optimal fit were 0.18 mMol acetyl-CoA/mg acidogen/day and 0.4 mMol/L respectively. Comparing these values to the kinetic parameters of pyruvate accumulation (Table 2-3) indicates that $\alpha << ß$ and $K_{s,PYR} >> K_{s,CoA}$. A higher value of ß and the corresponding lower value of $K_{s,CoA}$ means that acetyl-CoA is utilized as soon as it is produced. Hence, pyruvate accumulation should dominate acetyl-CoA accumulation. Assuming a pseudo-steady state for acetyl-CoA and accounting for pyruvate accumulation an optimal fit of the model to the experimental data was obtained. As expected, neglecting acetyl-CoA accumulation did not affect model predictions. Hence, the same figures (Figures 2-8, 2-9, 2-10 and 2-11) represent the best fit of the model to experimental acetic acid, propionic acid, butyric acid and methane production rate data.

Table 2-4 presents the normalized sum of the squared errors for the various fits. Relaxing pseudo-steady state assumptions for pyruvate yields a minimum value of 105 for the normalized sum of squared errors compared to a value of 148 allowing pseudo-steady state for pyruvate and acetyl-CoA. However, relaxing pseudo-steady state assumption for acetyl-CoA does not improve model performance.

Table 2-4
Comparison of the optimal normalized sum of squared errors between the three proposed mechanisms

Pseudo-steady state assumption for pyruvate and acetyl-CoA	148.3
Pseudo-steady state assumption only for acetyl-CoA and allowing accumulation of pyruvate	105.1
Relaxing pseudo-steady state assumption for pyruvate and acetyl-CoA	106.0

The steady state model predictions are compared to experimental data in Table 2-5. The model does well in calculating the methane production rate, methane content of the gas phase and volatile organic acid concentrations. To determine the experimental washout HRT, the dilution rate was stepped up from the normal value and the digester was operated at the new value until the methane production rate reached a steady state. Then the procedure was repeated by stepping up the dilution rate. It was found that washout occurred between 10 and 12 days residence time which is in agreement with the model prediction of 11.2 days.

CONCLUSIONS

A mathematical model for a glucose fed continuously stirred anaerobic digester was developed. The model incorporates kinetic expressions for all the major bacterial species involved in the anaerobic digestion process and also physico-chemical relationships in the liquid and gas phases. The bacterial populations are assumed to be inhibited by hydrogen which is in agreement with the current knowledge of the process, where it is believed that hydrogen accumulation causes build up of propionic and butyric acids. The model is capable of predicting essential design parameters like gas production rates, gas composition and pH of liquid phase. The performance of the model in predicting volatile organic acid build-up and rise in methane production rate when the digester was subjected to a feed overload is evaluated. It was found that relaxing pseudo-steady state assumptions for pyruvate

greatly improves model performance. However, no further improvement is achieved by allowing for acetyl-CoA accumulation.

Table 2-5
Comparison of experimental results during normal digester operations to model predictions

Methane production rate (L/day/L reactor volume)	0.67 - 0.7	0.68
Methane percent (%)	57	53.8
pH	7.1 - 7.2	7.1
Total biomass (g/L)	4.0 - 5.0	4.0
Acetic acid (mg COD/L)	10 - 50	15.47
Propionic acid (mg COD/L)	10 - 40	20.46
Butyric acid (mg COD/L)	< 10	3.28
Washout HRT (days)	10 -12	11.2

CHAPTER 3
CONTROL STRATEGY TO PREVENT IMBALANCE OF ANAEROBIC DIGESTERS DUE TO INHIBITORS ENTERING WITH THE FEED

Introduction

Biological conversion processes can fail when exposed to compounds that are toxic to the microorganisms. The effect of a toxicant on aerobic or anaerobic bacteria has been shown to be equally severe (Blum and Speece, 1992). However, in anaerobic digestion if the methane bacteria are inhibited by a toxicant, the result is an accumulation of volatile organic acids leading to a "soured" digester. Due to the prolonged generation times of the acetoclastic methanogens, the digester may have to be shut down for months to repopulate it with a healthy culture. Common inhibitors of anaerobic waste treatment processes are oxygen, heavy metals and aromatic compounds like phenol. Some of these inhibitors imbalance the process even when present in trace concentrations. Presence of traces of oxygen can cause failure. The methanogenic bacteria are obligate anaerobes. It is essential that a highly reduced environment be maintained to promote their growth. Dirasian *et al.* (1963) found that optimum digestion occurred with an oxidation-reduction potential between -520 and -530 mV. Converse *et al.* (1971) found that at oxidation-reduction potentials greater than -360 mV, methane production was completely inhibited.

Heavy metal ions inhibit or kill microorganisms by inactivating a wide range of enzymes. The heavy metal ions react with the sulfhydryl groups on the enzymes. Toxicity due to cuprous, cupric, zinc, ferrous, nickel and cadmium ions has been studied by several researchers. Mosey and Hughes (1975) showed that 163 mg/L of zinc, 180 mg/L of cadmium, 170 mg/L of cupric or 1750 mg/L of ferric ions can cause failure of digestion. Though, Jarrell *et al.* (1987) reported that even 10 mg/L of zinc or cuprous ions can cause 50% inhibition of the methanogenic bacteria. Phenol is toxic and is known to cause inhibition of the anaerobic digestion process (Field, 1989). Phenol is a monomer of polymeric compounds like tannins, humous and lignin. It is also present in organic bactericides, pesticides and the amino acid tyrosine. Waste streams from paper and pulp mills, food industries and synthetic chemical processes also contain phenol. Chou *et al.* (1978) reported that 2444 mg/l of phenol causes 50% reduction in activity of unacclimated methanogens. The examples presented above indicate that even low concentrations of the above toxicants are potent enough to cause inhibition. The toxicants lower the maximum specific growth rate of the methanogens. If this growth rate drops below the dilution rate then wash-out occurs. Therefore, control measures should be taken as soon as an inhibitor enters the digester so that it does not build up. The digester can be saved if the dilution rate is lowered below the ultimate value of the methanogen specific growth rate. The question is how should the dilution rate be lowered.

This chapter details the theoretical development of a control law, using a model for the process, that prevents imbalance of digesters when it is exposed to an

inhibitor through the feed. The variable that was manipulated so as to mitigate the severity of an inhibitor was the dilution rate. Methane rate was used as the measured variable since it is easily measured on-line.

The Optimization Problem

Since digester failure results in cessation of methane production and since the latter is the valuable product and can be relatively easily measured on line, a reasonable performance measure to be maximized is

$$J(D(t)) = \int_0^{t_f} Q_{methane}(t)\, dt \qquad (3-1)$$

where $Q_{methane}$ is the methane production rate, D(t) the dilution rate and t_f the final time ($\geq$ 3 time constants). To have a tractable optimization problem the dynamics of all reaction steps except for the rate limiting one (growth of methanogens and associated production of methane) are neglected. Also, by assuming that the feed substrate concentration does not vary, stoichiometry can be used to express volatile organic acids concentration in terms of methanogen concentration. Then the state variables are reduced to two, the methanogen concentration X and the inhibitor concentration I. Neglecting inhibitor consumption the model equations are

$$\frac{dX}{dt} = -DX + \mu(X,I)X \qquad (3-2)$$

$$\frac{dI}{dt} = D(I_o - I) \qquad (3-3)$$

where the specific growth rate is

$$\mu(X,I) = \frac{\mu_{max} f(I)}{1 + \frac{K_s}{S_o - \frac{X}{Y_s}}}$$

The function f(I) accounts for the inhibitor effect on the growth rate; two functional forms were considered

$$f(I) = e^{-aI} \qquad (3-4)$$

$$f(I) = \frac{1}{1 + bI} \qquad (3-5)$$

The methane production rate is proportional to the methanogen growth rate

$$Q_{CH_4} = VY\mu(X,I)X \qquad (3-6)$$

Optimization

The Hamiltonian is linear with respect to the manipulated variable, D(t), and therefore for all t the optimal D is either on a singular arc or on a bound. Using a

brute force approach, the gradient projection method (Kirk, 1970), it was established that the optimal D(t) starts on a bound (either zero or D_{max}, depending on the initial conditions) until it hits a singular arc and stays on the singular arc until the final time (t_f). Computer memory limitations did not allow the use of this approach when t_f was large. However, a different approach was used.

A unique expression for D on the singular arc in terms of the states and costates were obtained by setting the second time derivative of the partial of the Hamiltonian with respect to D equal to zero. The expression for D was then substituted into the state and costate differential equations to obtain a system of four differential equations that describes the singular arc. Since the work with the gradient projection method determined that at t_f, D was on a singular arc and since the optimal steady state value of D was not on a bound it was concluded that for large t_f the final values of the state variables are very close to their optimal steady state values. Pontryagin's maximum principle (Kirk, 1970) gives the final values of the costates as zero. With this information the state and costate differential equations were integrated backwards in time to determine the singular arc. Depending on the initial values of the state variables, the singular arc can be reached by operating for a short period of time either batch or at the maximum dilution rate. Figure 3-4 shows the singular arc trajectory. Initially the optimal operation would be to stay batch and then move along the singular arc. In this manner the optimal control law was determined.

Optimal and Easily Implementable Suboptimal Control Laws

Figure 3-1 depicts a typical optimal dilution rate for the case where inhibitor enters at time zero and its effect on specific growth rate is given by equation 3-4. The optimal response is to operate the digester in a batch mode for less than 0.006 days and subsequently change the dilution rate according to the equations of the singular arc. Similar results are obtained with inhibitors following equation 3-5. In practice, given that the model is not accurate and that the feed stream is not characterized on line (e.g. I_o is not known) the above "optimal" control law cannot be implemented. For this reason a good suboptimal easy to implement control law was sought.

An inspection of Figure 1 shows that almost for the entire time interval of optimization the dilution rate was given by the singular arc expression. If this dilution rate is plotted against the corresponding methane production rate, the result is a straight line (Figure 3-2). The same straight line is obtained if the inhibitor effects are given by equation 3-5. Thus the control law on the singular arc is to simply change the dilution rate in proportion to the methane production rate (which can be measured on line), i.e.,

$$D(t) = kQ_{CH_4}(t) \qquad (3-7)$$

A value very close to the optimal value of the proportionality constant can be easily determined experimentally since under normal operating conditions virtually all the

substrate is converted to methane carbon dioxide. Thus one can take as k the dilution rate to methane rate ratio under normal operating conditions. Equation 3-7 is not only easily implementable but is also a very good suboptimal control law as seen from Figure 3-3.

Conclusions

An optimal feed-back control law that manipulates the dilution rate so as to prevent imbalance of the anaerobic digestion process was developed using Pontryagin's maximum principle on a simplified version of the dynamic anaerobic digestion model that was presented in the previous chapter. The optimal control law is a function of the state variables methanogen and inhibitor concentration. It is impossible to implement the control law because these variables cannot be measured. However, it was found that if the dilution rate is varied in proportion to the methane production rate then the performance of this sub-optimal control policy was close to that of the optimal control policy. The sub-optimal control policy can be implemented real-time on a anaerobic digester because methane production rate can be easily measured on-line. The usefulness of this policy in preventing imbalance to the process will be demonstrated in the subsequent chapter.

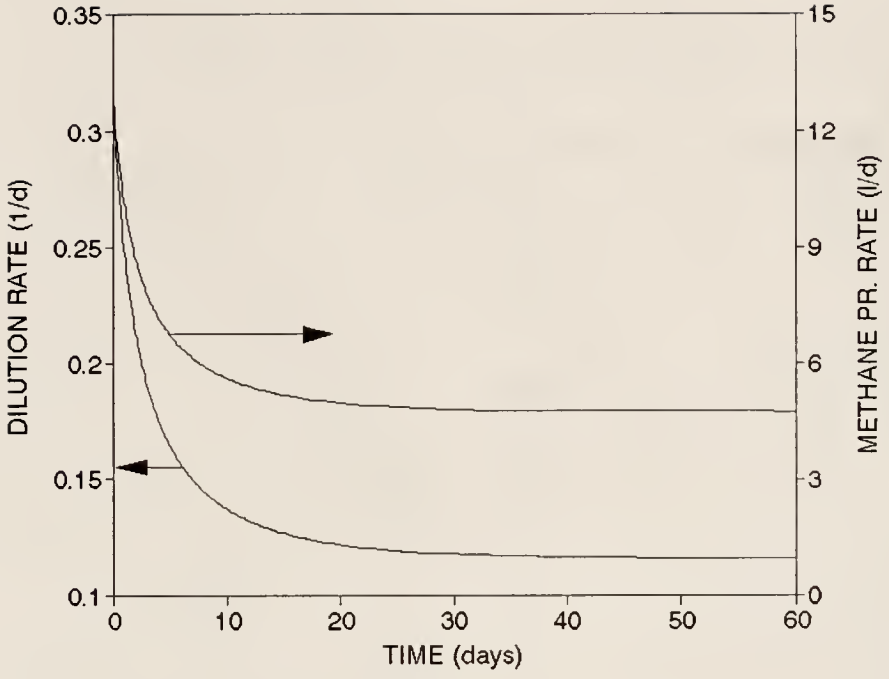


Figure 3-1 : Optimal D(t) and corresponding methane rate versus t.

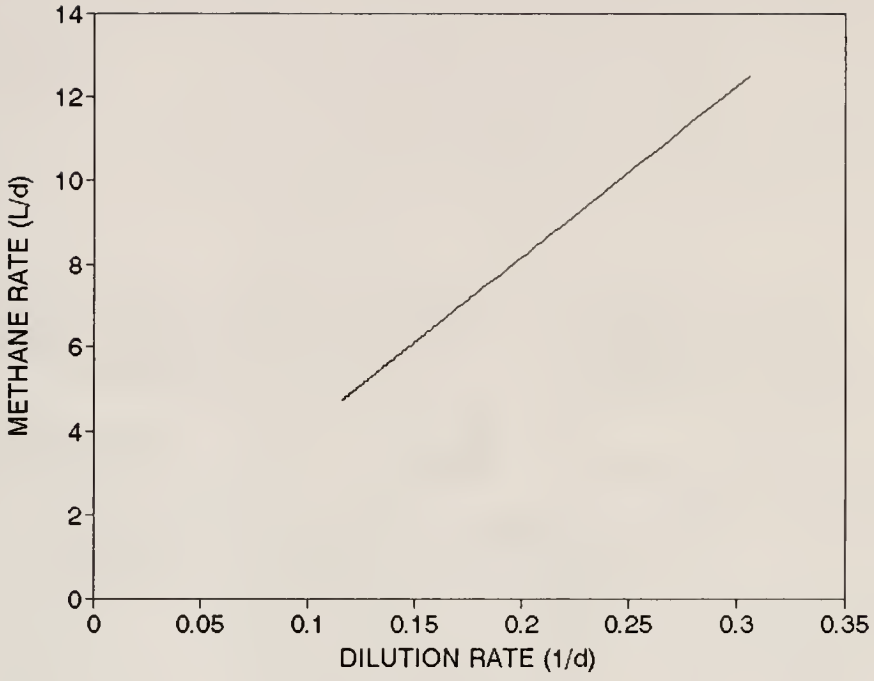


Figure 3-2 : Methane rate versus D on the singular arc with f(I) given by either equation 3-4 or equation 3-5.

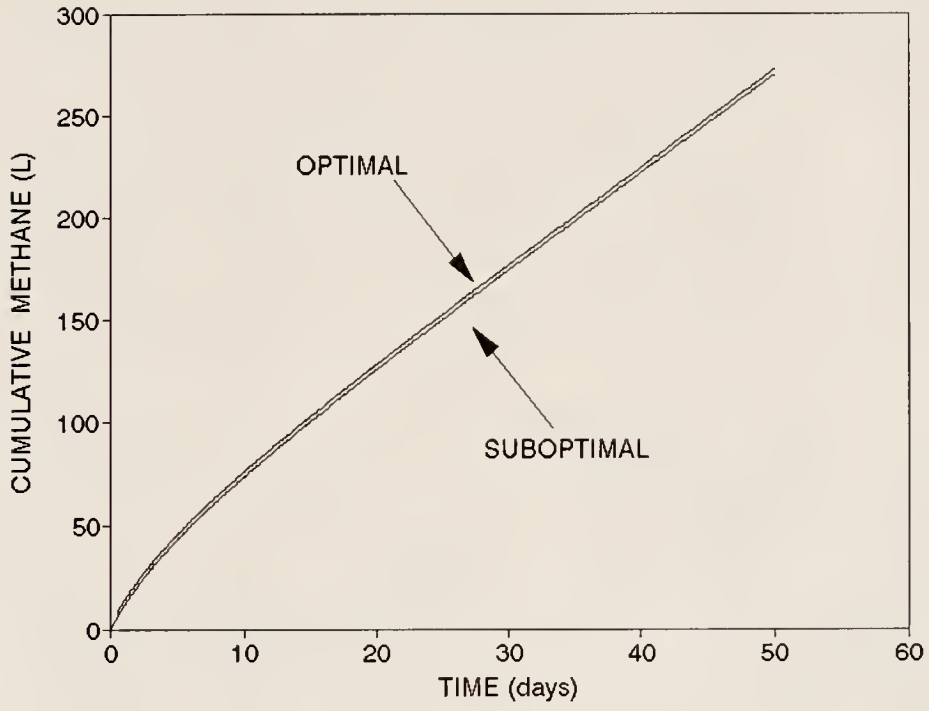


Figure 3-3 : Cummulative methane versus time. Suboptimal line is calculated using equation 3-7.

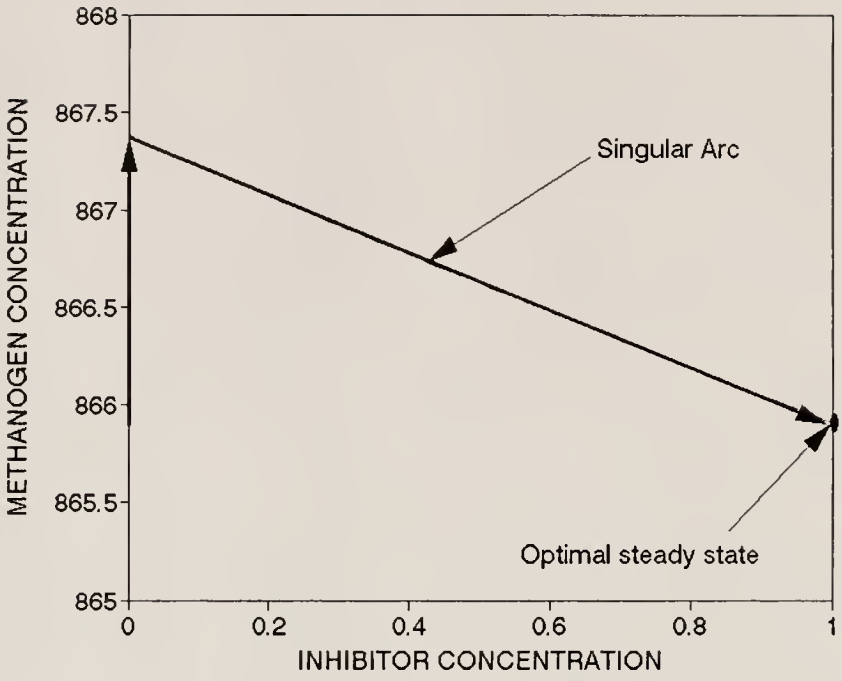


Figure 3-4: The singular arc

CHAPTER 4
AN EXPERT SYSTEM FOR THE CONTROL OF ANAEROBIC DIGESTERS

Introduction

The importance of the need for an on-line control scheme for the anaerobic digestion process was emphasized in an earlier chapter. Several investigators have developed approaches to control the process (Podruzny and van den Berg, 1984; Russell *et al.*, 1985; Rozzi *et al.*, 1985; Whitmore and Lloyd, 1986; Renard *et al.*, 1988; Denac *et al.*, 1988, Dochain *et al.*, 1991). The existing control strategy in the industry is to rely on pH control as a means of ensuring smooth digester operation. Rusell *et al.* (1985) describes the installation of automatic controls on a UASB treating waste from a potato starch manufacturing industry. The plant was equipped with pH, temperature and flow control units. The pH was maintained between 6.8-7.6. However, pH is not a reliable control variable because changes in pH occur long after the onset of an imbalance. Automatic control schemes have been proposed for the control of bench scale and pilot scale digesters. Podruzny and van den Berg (1984) developed a computer controlled system for a down-flow stationary fixed film digester. The control variable used was gas production rate. The control scheme involved maintaining the gas production rate at a set point through a proportional-integral controller by manipulating the feed rate. When the gas

production dropped due to inhibition and the controller was not able to maintain set-point, then the set-point was arbitrarily dropped by 40%. There is a major drawback in using gas production rate as a control variable because its response to inhibitors is inconsistent. Total gas production rate is the sum of methane, carbon dioxide and hydrogen production rates. Most of the hydrogen is utilized by the hydrogen utilizers where one mole of carbon dioxide and four moles of hydrogen are converted to one mole of methane. Hence, due to this reaction the gas volume decreases from five to one. However, if the hydrogen utilizers are inhibited the above conversion reaction does not proceed resulting in an increase in the gas production rate. If the inhibitor were to inhibit the growth rate of all bacterial species then the result would be a drop in gas production rate. Moreover, during inhibition dropping the set-point by 40% may not be sufficient. Automatic control based on other variables has been developed for laboratory scale digesters. Whitmore and Lloyd (1986) developed a membrane inlet mass spectrometer unit to detect liquid phase hydrogen. They propose a control scheme where the liquid phase hydrogen concentration is maintained at a certain level. They tested the efficacy of this control scheme against feed overloads. However, this control scheme will not work when the digester is exposed to an inhibitor like phenol. Phenol is toxic to all populations of bacteria. Hence phenol would inhibit even the acidogenic bacteria. This would cause hydrogen concentrations to drop, in response to which the above control scheme would increase loading rate, thus worsening the problem. Moreover, it may not be economical to dedicate a mass spectrometer for on-line analysis of just hydrogen.

However, using liquid phase hydrogen concentration as a control variable is an attractive concept. Renard *et al.* (1988), developed an adaptive control algorithm for a continuously stirred tank digester using digester substrate concentration as the control variable. This controller was tested successfully in preventing imbalance due to feed overloads. It should be pointed out that the adaptive controller also relied on the availability of feed COD data. This information may not be readily available since it is not measurable on-line. The problem is worsened if there are wild fluctuations in feed COD. The studies mentioned above did not consider the possibility of process failure due to inhibitor entering with the feed. Such a type of inhibition complicates considerably the control problem.

An expert system for preventing imbalance of continuous glucose fed digesters is presented here. It addresses both the feed inhibition and overload problem. The control scheme proposed here uses methane production rate as the principal measured variable and dilution rate as the manipulated variable. Methane production rate is not only a readily measured quantity, but also has the advantage of providing an early indication of digester imbalance as compared to other indicators like pH depression or volatile organic acid accumulation. Conventional control schemes designed to reject a disturbance due to feed overload can fail during feed inhibition because sign reversal in the steady-state gain. Under normal operating conditions an increase in the dilution rate would result in an increase in the methane production rate. Thus the sign of the steady-state gain is positive. In case of feed inhibition, however, increasing the dilution rate would add more

inhibitor to the digester thus increasing the inhibitor concentration in the reactor, something which could possibly drop the methane production rate further; thus the sign of the steady-state gain becomes negative. Hence, the control algorithm should be able to recognize gain reversal and implement appropriate control laws. An expert system was developed to accomplish this.

The expert system switches between a conventional set-point control law and the constant yield control law developed in the previous chapter depending on the sign of the steady state gain. Disturbances studied were feed overload, feed underload and an inhibitor (phenol) entering with the feed. Methane production rate was used as the measured variable. Dilution rate was the manipulated variable. Subsequent sections detail the development of the expert system, simulations of the expert system using the model developed in Chapter 2 and finally experimental validation of the expert system.

Control Strategies

In this section the two main components of the expert system are briefly described.

Conventional Set-point Control law

Under normal conditions the digester is under conventional set-point control. The set point to be maintained is the methane production rate obtained when the reactor was run at a moderate dilution rate (e.g. 0.05 $days^{-1}$) with normal feed

concentrations. Since overloads are accompanied by an initial increase in the methane rate, a conventional positive-gain control law responds by lowering the feed rate and thus lowering the volatile organic acid concentration in the reactor. This prevents organic acid build-up and saves the digester.

Constant Yield Control Law (CYCL)

Conventional positive-gain set-point control would have adverse effects if implemented when the process gain has been reversed due to an inhibitor entering with the feed. In the previous chapter it was shown that a control law that changes the dilution rate D in proportion to the methane production rate Q, i.e.

$$D(t) = k\, Q_{methane}(t)$$

closely approximates the optimal response to noncompetitive feed inhibitors of the methanogens. If the active volume of the digester does not change, the above policy keeps the volumetric yield (ratio of the volume of methane produced to the volume fed) constant. A value very close to the optimal value of the proportionality constant, k, can be easily determined, since under normal operating conditions virtually all of the substrate is converted to methane and carbon dioxide; thus k can be taken as the ratio of dilution rate to methane production rate under normal operating conditions. It should be observed that the constant yield control law has negative gain, thus it is appropriate when the process gain changes sign. Harmon *et*

al. (1990) first suggested the use of this strategy to prevent imbalance resulting from temperature changes.

Key features of the Expert System

Some of the key features of the expert system are listed below

1. Methane production rate is used as the sole on-line measured variable.
2. A statistical criterion is used to quantify whether the output increases or decreases.
3. Automatic selection of control law.
4. Smooth transfer between control laws (bumpless transfer).

Methane production rate is the on-line measured variable. Decisions regarding the selection of the control strategy are based on the absolute value of this variable and also the rate of increase or decrease of the variable. A t-test is used on past sampled methane production rate data to test whether the hypothesis, "the rate of change of the methane production rate is negative," can be accepted with a certain degree of confidence. The t-test is done only when the methane production rate has dropped below a minimum bound. Depending on the acceptance or rejection of the hypothesis an appropriate control law is chosen. Before switching from set-point control law to CYCL the digester is operated batch and a switch from CYCL to set-point control law is done only after the methane production rate has reached the set-point. These two switching features result in bumpless transfer between control

policies. By operating batch, it is ensured that excess substrate in the digester is consumed and the CYCL initially implements a low value for the dilution rate.

The Expert System

The expert system is outlined in the flow diagram of Figure 4-1. As long as the methane production rate (CH_4) remains in a "normal" range $CH_{4min} < CH_4 < CH_{4max}$ the digester is operated using a conservatively tuned set-point controller. The controller must be conservatively tuned because large increases in the dilution rate can negatively affect the culture. A choice we have been using successfully is internal model control (IMC; Morari and Zafiriou (1989)) with controller tuning based on an experimental step response curve. The "normal" range should be chosen so as to include variations due to measurement noise and due to the initial process response to the frequently encountered mild disturbances that the set-point controller can easily handle. We have been using as "normal" range ±20% of the methane set point. If the methane production rate rises past CH_{4max} then the cause is attributed to a significant feed overload. To avoid running the risk of being too late in decreasing the dilution rate (the set-point controller is conservatively tuned), the algorithm first sets the dilution rate to a very low value D_{low} (e.g., 0.001 $days^{-1}$), thus ensuring that the sluggish set-point controller approaches the new dilution rate from below.

A drop in the methane rate to below CH_{4min} is due to a severe underload or to feed inhibition. Gain sign reversal is a possibility. If this is not the case, the set-point controller, by increasing the dilution rate (slowly and only up to a maximum value below the maximum specific growth rate of the acid utilizing methanogens (0.37 $days^{-1}$, according to the model of Chapter 2), will increase the methane rate and may eventually return the system to the set point. If, however, the process gain sign has been changed the digester will respond to increases in the dilution rate by further lowering of the methane rate. If the methane drops below the minimum bound, then a t-test is performed on the last 30 data points to statistically determine whether the methane rate is indeed dropping. If it is dropping then the algorithm recognizes a reversal in the sign of the gain and exits the set-point control loop.

The preceding digester operation at a high dilution rate might have caused some of the biomass to wash out and/or accumulation of hydrogen which is inhibitory. Hence, before implementing the constant yield control law (CYCL), which is appropriate when the gain reversal has taken place, the digester is operated batch for an interval of time. An initial batch interval is also suggested by the optimal control law (Pullammanappallil *et al.*, 1991, Chapter 3) while the digester is operated in a batch mode hydrogen is generated by the conversion of propionic and butyric acids to acetic acid and utilized by the hydrogen-utilizing methanogens. Once the levels of propionic and butyric acids drop the hydrogen concentration will rapidly decrease to noninhibitory levels. Thus, if propionate and butyrate measurements are available the batch phase is terminated when the concentrations of these organic

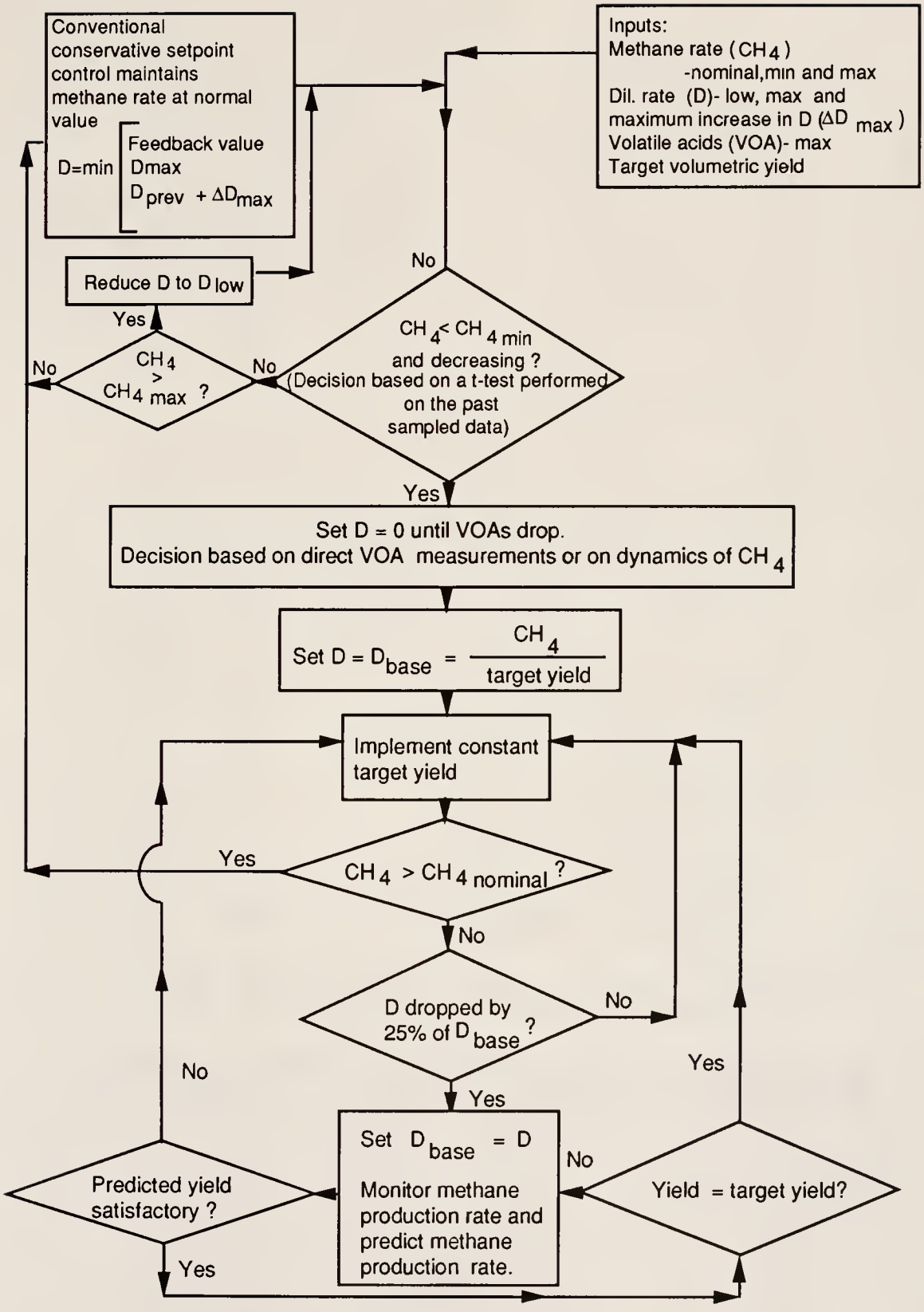


Figure 4-1 : The Expert System.

acids drop to close to their normal levels (e.g., to less than 200 mg COD/L for the sum of the two. If propionate and butyrate measurements are not available the duration of the batch period can be decided from the following reasoning: When the reactor goes batch the feed inhibitor concentration stops increasing. Due to dynamics the rate of methane production may continue to drop for a short interval of time (e.g. 8 hrs). Subsequently, if hydrogen is at high levels its consumption will reduce its inhibition and as a result the methane rate will start rising. Eventually, the methane rate will start dropping due to reduced substrate levels. Thus the decision to terminate the batch phase can be based on when a downwards trend of the methane production rate is confirmed. The recommended procedure then is, after a short minimum interval (8 hours), to stop the batch phase when two measurements are obtained that are by more than two measurement noise standard deviations below the maximum value of the methane rate in the batch interval. A statistical test involving more data points is not used since with our experimental set-up the frequency of methane rate readings is very low at low methane rates.

After the termination of the batch phase the algorithm enters a region in which the primary control law is the previously described CYCL, according to which the dilution rate changes in proportion to the methane rate so as to maintain the nominal volumetric yield. This law is well suited for responding to feed inhibitors. In rare instances, however, the gain sign reversal that brought the algorithm to the CYCL might have been caused by a very severe underload that dropped the methanogen specific growth rate to below the dilution rate. Then the nominal

volumetric yield cannot be maintained and the CYCL will keep decreasing the dilution rate towards zero. To prevent this, if the dilution rate drops to 75% of the value at the start of the CYCL (D_{base}), it is kept at that value for five sampling intervals. Methane production data obtained during this period are then used to predict whether or not the methanogens are washing out. Extrapolation is carried out with a second order polynomial fit and an exponential fit. If the predicted minimum methane rate using either extrapolation methods is less than 10% of the normal methane production rate, then the digester is switched back to the CYCL. Otherwise the digester will continue being maintained at a constant dilution rate until either washout is predicted or until the volumetric yield increases to its nominal value. In both cases then the algorithm switches to the CYCL. In case the methane rate is dropping, the CYCL will reduce the dilution rate until the methane rate stabilizes. If the feed conditions return to normal then the CYCL will increase the dilution rate in response to the increase in the methane production rate, thus moving the digester towards the set point. When the methane rate reaches the set point, the algorithm switches to set-point control. An earlier switch is risky because the CYCL may be operating at a dilution rate only slightly less than the methanogen specific growth rate.

Materials and Methods

The expert system was tested on a laboratory scale 1.7 liter digester. This set up was different from the one described in chapter 2. This experimental setup is

shown in the schematic diagram of Figure 4-2. The digester is equipped with on-line measurement of the methane production rate and computer control of the dilution rate. The entire setup is interfaced to an IBM compatible personal computer. The feed is pumped through a computer controlled peristaltic pump equipped with a second pump head to withdraw effluent. Hence, effluent is withdrawn at the same rate as the feed is pumped. The gas vented from the digester is scrubbed off its carbon dioxide content by passing it through a column of soda lime. The methane accumulated in one limb of the U-tube by displacing the liquid. When the liquid in the other limb rose to a certain level, a float switch was tripped, which caused two events to happen simultaneously. A signal was sent to the computer and also the methane from the first limb was exhausted resetting the liquid level. By measuring the time between two successive signals and given that the volume of methane that triggers the signal is known, the methane production rate could be calculated.

Normal operating conditions are a dilution rate of 0.05 $days^{-1}$ and a digestion temperature of 35 °C. The feed was a glucose medium, the nominal concentration of glucose was 28.8 g/L. The composition of other nutrients is given in Appendix B. While making a feed overload or an underload only the glucose concentration was changed. Sodium propionate was added to the feed so as to always maintain a culture of propionate degrading bacteria in the digester. During normal operating conditions the methane production rate varies between 1.0 L/day and 1.3 L/day..

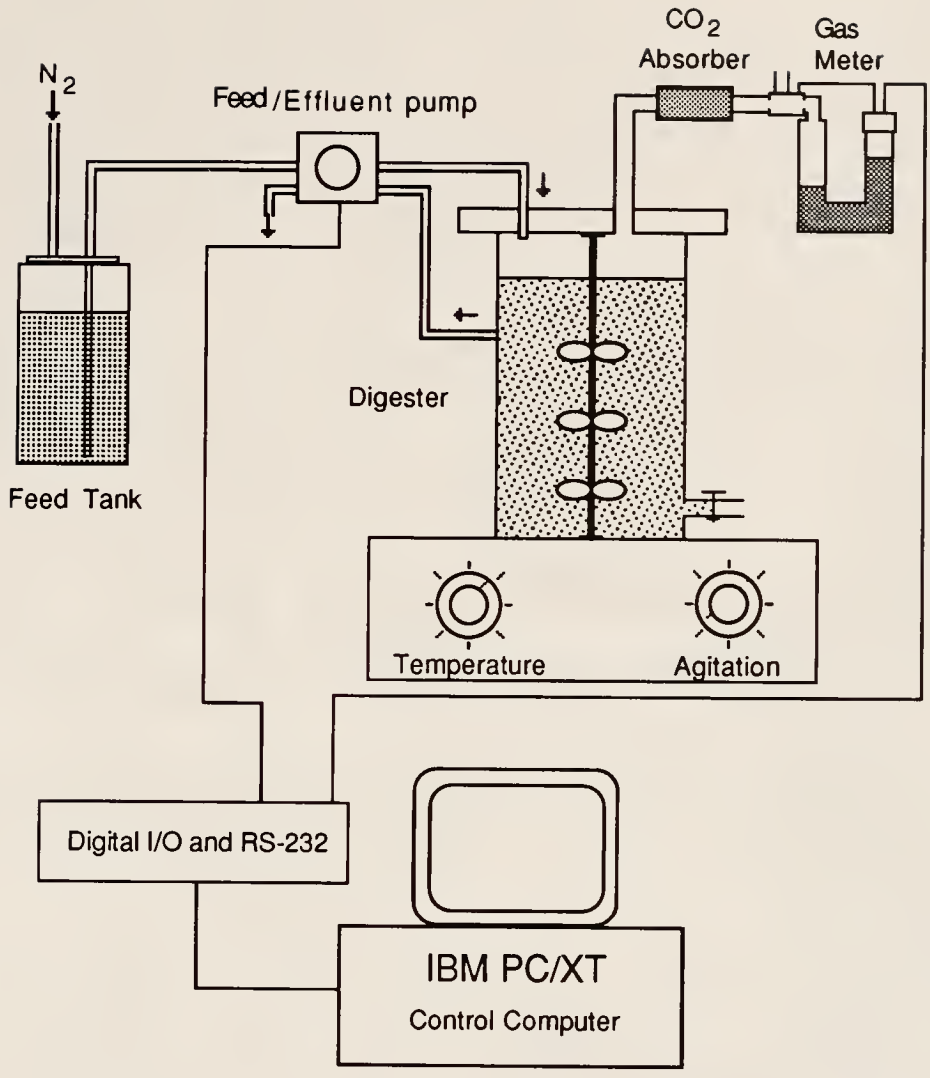


Figure 4-2 : The bench scale digester.

Testing the Expert System

Simulations

Computer simulations were done to test the expert system before it was actually implemented on the laboratory scale digester. The anaerobic digester was simulated using the model that was developed in Chapter 2. The efficacy of the expert system in rejecting a disturbance was tested for a feed underload, feed overload and inhibitor entering with the feed.

Underload and overload. Figure 4-3 shows the expert system response to a feed underload and feed overload. The glucose concentration in the feed was reduced from 28.8 g/L to 20.8 g/L and then to 15.8 g/L. In response to the underload the methane production rate dropped, hence the controller increased the dilution rate to maintain the set point. No adverse effects, like steady state gain reversal were created by this, so the controller continued to operate the digester at the higher dilution rate. The controller responded in the same manner when the glucose concentration was dropped again. Total concentration of volatile organic acids (VOAs) remained below 100 mg COD/L.

An overload was simulated by returning the glucose concentration in the feed from 15.8 g/L to 28.8 g/L. The controller dropped the dilution rate so as to maintain set point. The nominal value of 0.05 d^{-1} was reached from below. The volatile organic acids plot shows that the expert system succeeded in preventing any accumulation of volatile organic acids.

Inhibitor entering with the feed. Figure 4-4 depicts the response of the expert system to an inhibitor entering with the feed. The inhibitor was assumed to affect only the growth rate of acetoclastic methanogens, following the kinetics of noncompetitive inhibition. In particular the maximum specific growth rates of the methanogens were multiplied by $1/(1 + 1000\ [I])$, where [I] denotes inhibitor concentration. Due to the inhibitor the methane production rate dropped and the controller initially increased the dilution rate so as to maintain set point. This action of the controller fed more inhibitor to the digester, thus worsening the problem. So the methane production rate continued to drop. When any further increase in dilution rate caused the methane production rate to drop, the controller recognized that the steady-state-gain sign had reversed so it changed the control scheme from set-point control to constant yield control law after operating the digester batch for the minimal period of 8 hours. The inhibitor was removed from the feed after 12 hours while the digester was being operated in constant yield mode. Once the inhibitor was removed from the feed the inhibitor in the digester began to wash out and this increased the maximum specific growth rate of the methanogens which in turn increased the methane production rate. Since the implemented dilution rate is proportional to methane production rate, the dilution rate increased. It took over 1000 hours for the methane rate to approach its nominal set-point value. It should be noted that even though it took considerable amount of time to return the digester to its set point, this could not have been helped since the CYCL sets the dilution rate very close to the methanogen specific growth rate. An attempt to speed up this

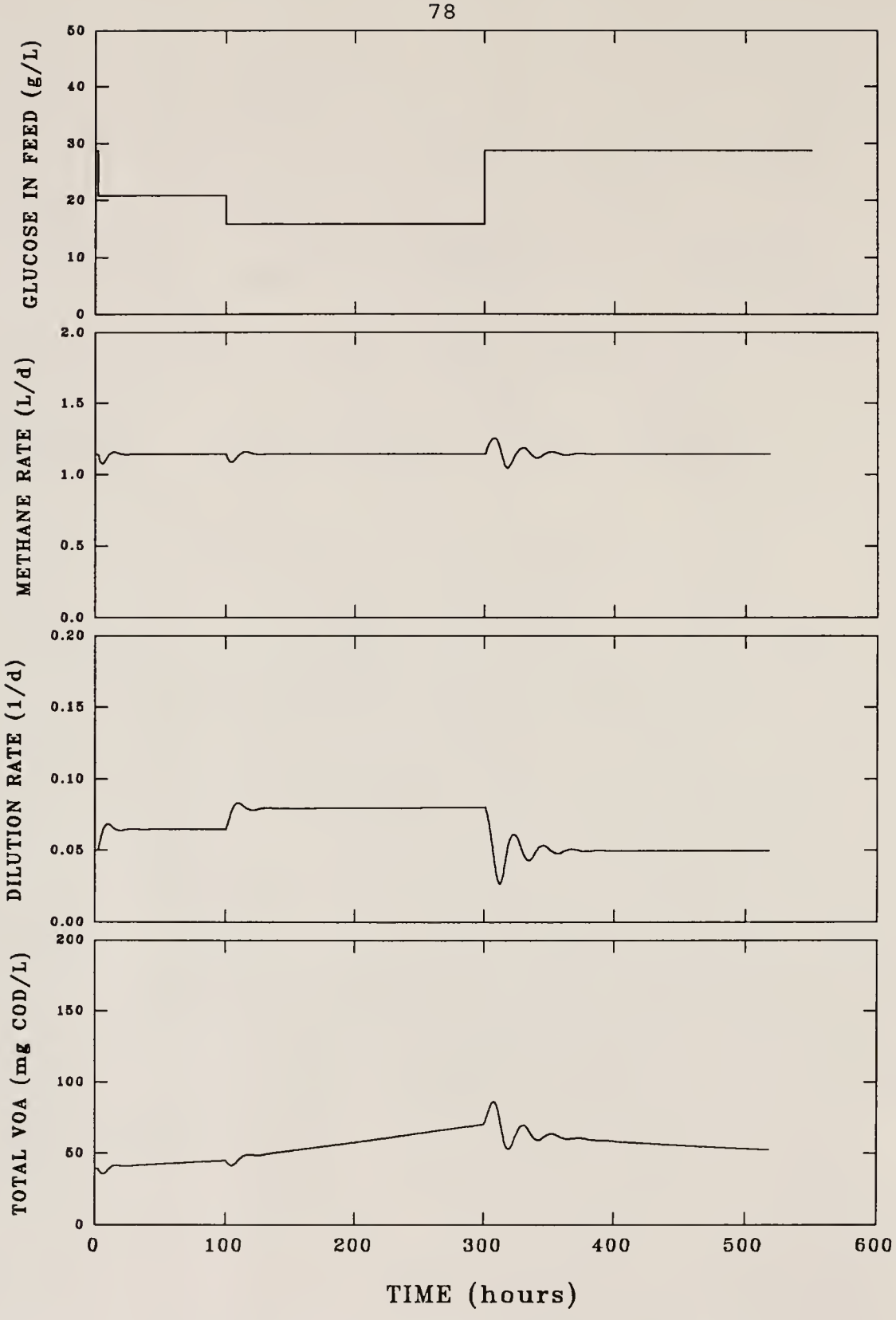


Figure 4-3: Simulation of the expert system response to an underload and an overload.

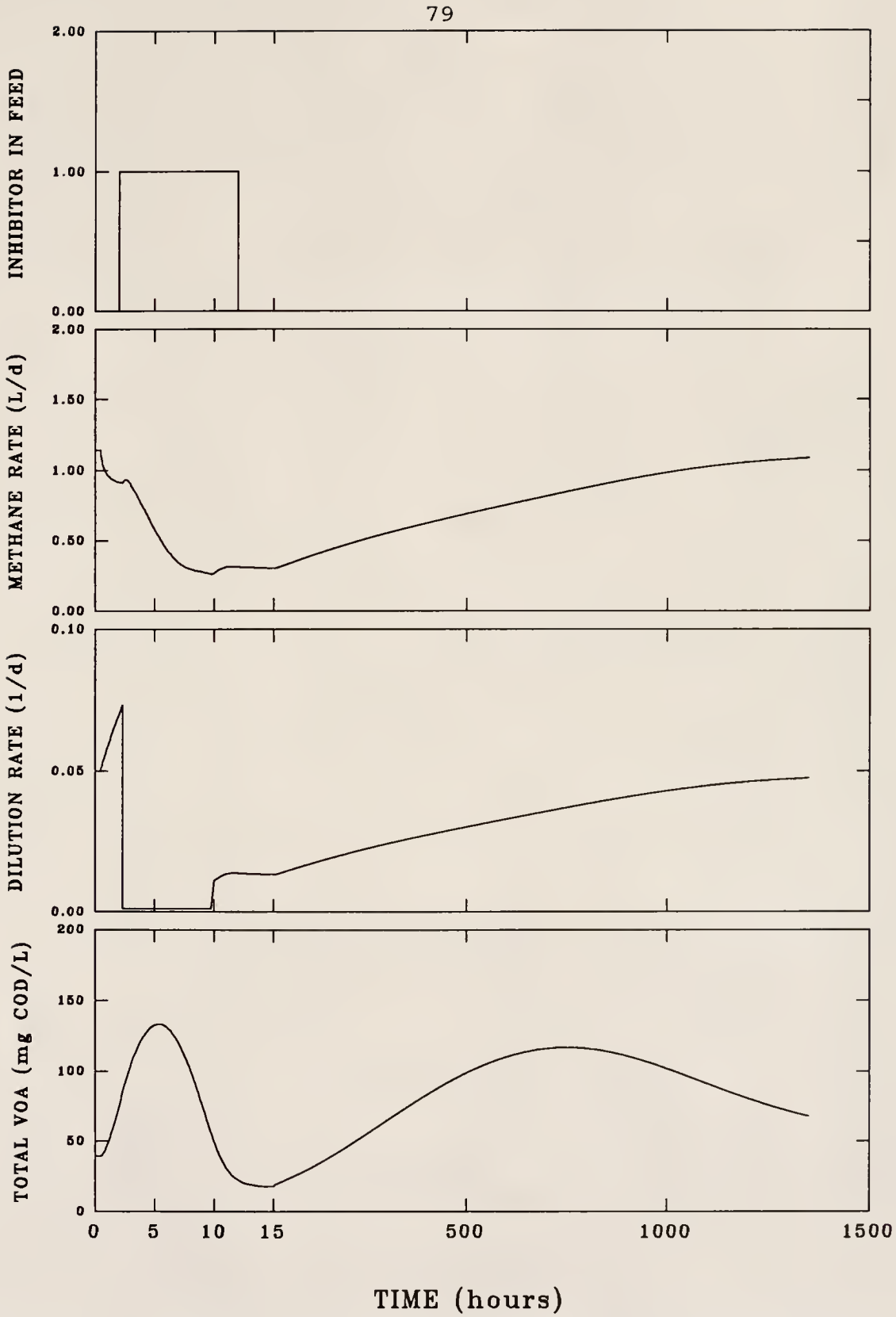


Figure 4-4: Simulation of the expert system response to an inhibitor entering with the feed.

return by increasing the dilution rate would have started washing out the methanogens and soon methane rate drop and VOA buildup would follow.

Experimental Validation

The expert system was implemented on the bench scale laboratory digester. The efficacy of the expert system in preventing digester imbalance when the digester was exposed to disturbances like a feed overload, feed underload and phenol entering with feed was tested.

Overload. Two tests were performed with an earlier slightly different version of the expert system. In this version the digester was operated at a constant dilution rate 0.05 $days^{-1}$, and the expert system kicked in only if the methane production rate increased or decreased by 20% of the normal value. Figures 4-5 and 4-6 show the response of the expert system to this disturbance. In both tests the glucose concentration in feed was stepped up to 86.4 g/L from 28.8 g/L for approximately 140 hours. The expert system entered set point control when the methane rate went past the maximum limit. When the glucose concentration was tripled the first response was the methane rate to increase. Since the methane production rate went above the upper bound, the controller decreased the dilution rate to 0.001 d^{-1} and the dilution rate was slowly brought up. The controller was able to maintain set point. The total volatile organic acid levels were kept below 300 mg COD/L. After the overload was removed the controller increased the dilution rate to maintain set point. The response of the expert system to both tests was similar.

Underload. The performance of the expert system to feed underloads was then studied. Three tests were carried out: a mild underload, a moderate underload and a severe underload. For the severe underload a slightly different earlier version of the expert system where the CYCL was implemented if the methane production rate remains below CH_{4min} for three consecutive sampling intervals, regardless of whether it was increasing or decreasing. In the present version of the expert system the constant yield policy is implemented only if the methane production rate is less than 80% of the normal value and is monotonically decreasing for three successive sampling intervals.

Figure 4-7 shows the expert system response to a mild underload where the glucose concentration was dropped from a nominal value of 28.8 g/L to 20.8 g/L. Low substrate concentration caused methane to drop. However, the controller was able to bring the methane rate back to the set point by increasing dilution rate from 0.05 d^{-1} to approximately 0.06 d^{-1} to maintain set-point. Figure 4-8 shows the expert system response to a moderate underload. The glucose concentration in the feed was halved. The response was similar to that of the mild underload. The dilution rate was increased to approximately 0.075 d^{-1} to maintain set-point. When the glucose concentration was brought back to normal then the dilution rate dropped close to 0.05 d^{-1}. Figure 4-9 shows the expert system response to a severe underload. No glucose was added to the feed, a 100% underload. The feed still contained casamino acids and yeast extract which could be used by the bacteria as source of energy. The glucose concentration was dropped at 10 hours and this was sustained

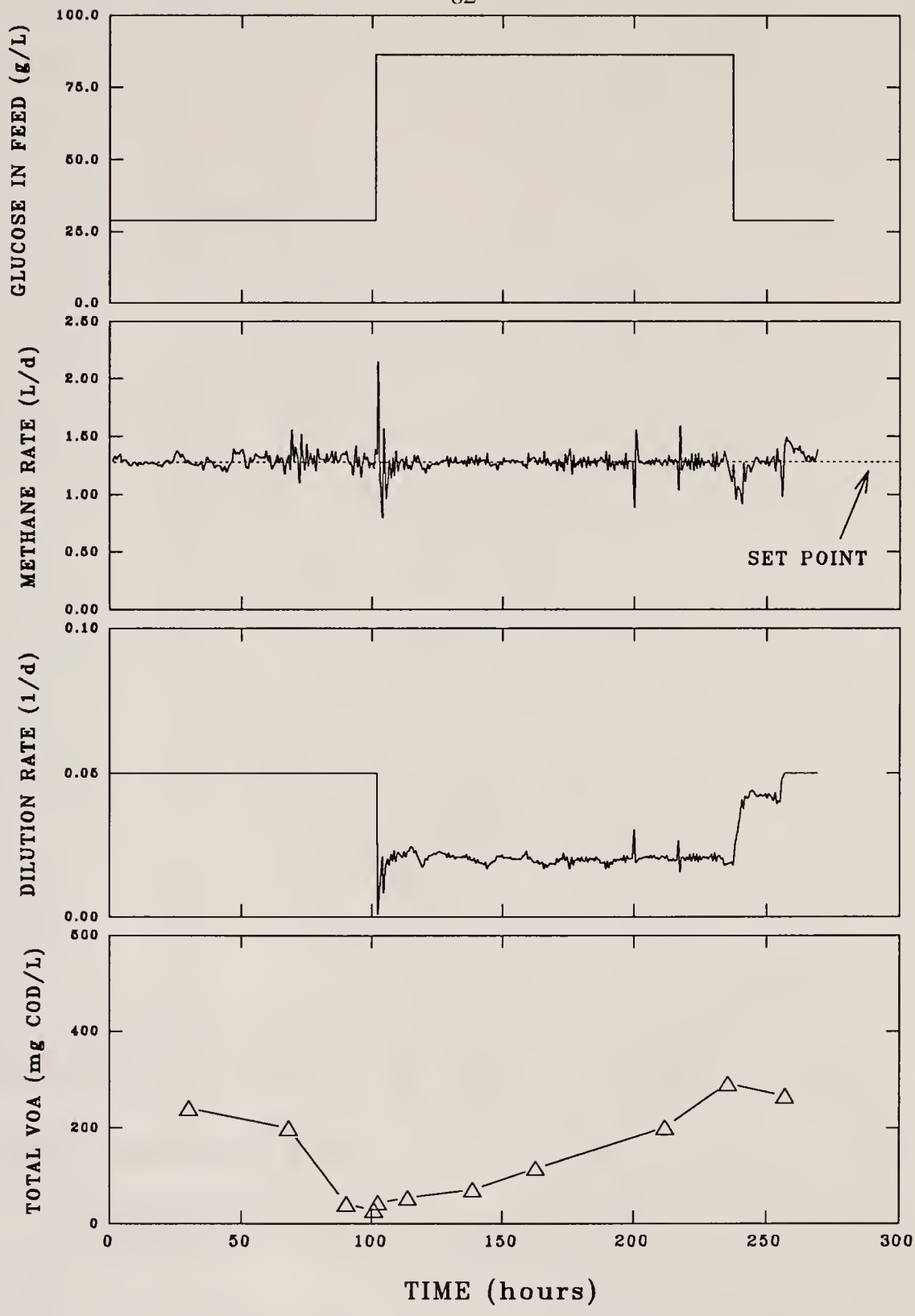


Figure 4-5: Experimental expert system response to a triple overload.

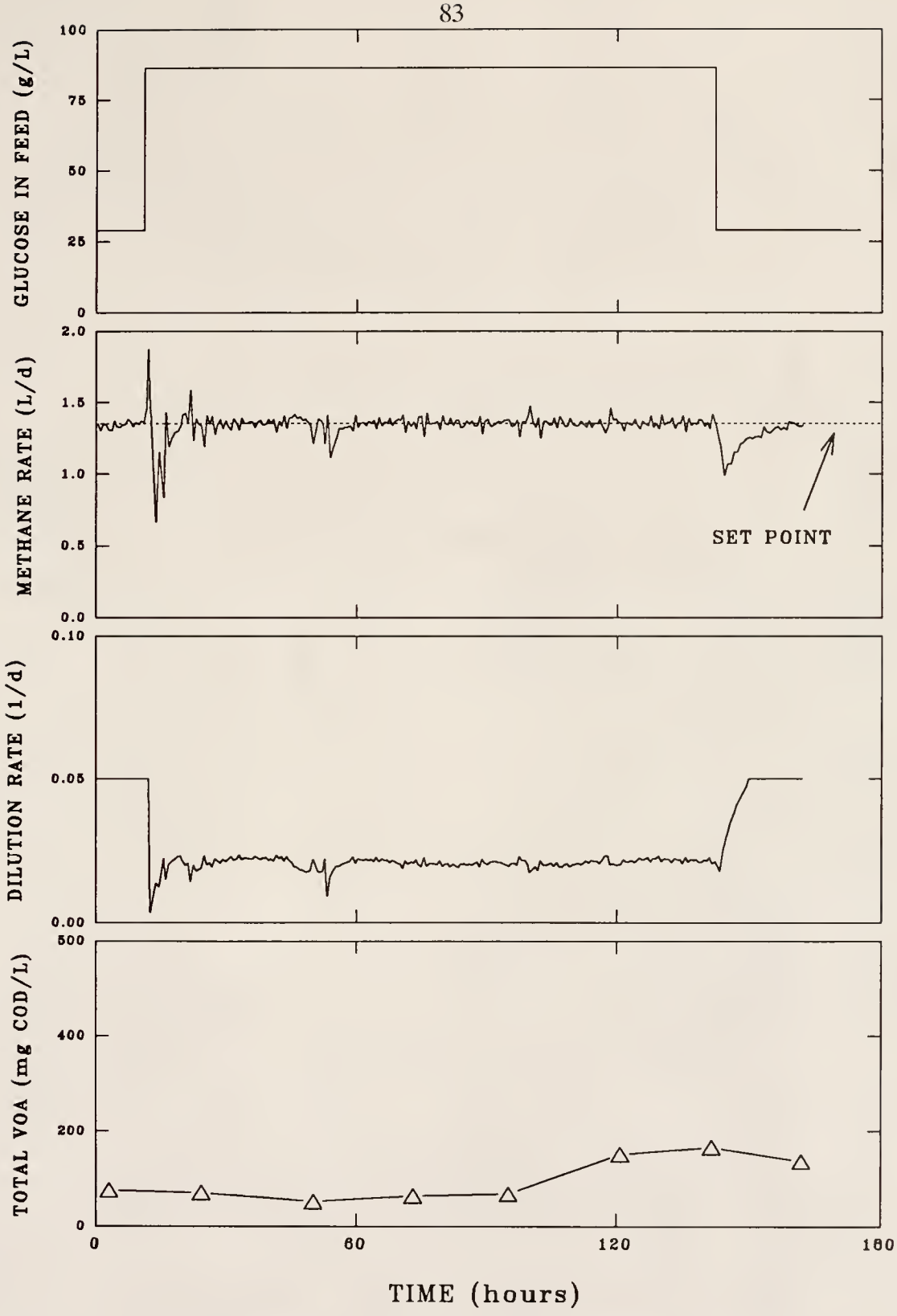


Figure 4-6: Experimental expert system response to a triple overload.

for 68 hours. The methane production rate began to drop and the controller increased the dilution rate but could not maintain set-point. Hence, the expert system concluded that the disturbance had caused a reversal in the sign of the steady state gain and implemented the CYCL after operating the digester batch for half a day. When the feed was switched to normal the methane production rate immediately increased above the upper limit. The expert system not only brought the digester into the set-point control mode but also sensed the response as having been caused by an overload, and so dropped the dilution rate to 0.001 $days^{-1}$. This drop in dilution rate caused the methane production rate to remain below CH_{4min} and once again the expert system went to the batch phase that precedes the CYCL. This response was unwarranted and would have been prevented if the present requirement of a monotonic drop had been incorporated into the expert system. Eventually, the CYCL brought the digester back to normal operating conditions (i.e a dilution rate of approximately 0.05 $days^{-1}$). The yield during batch operation is actually infinity. However, this phase is shown to be at a yield of 1000 L methane/L feed in the yield plot of Figure 4-9. Volatile organic acid concentrations are not shown for any of the underload experiments because these remained below detectable levels of the GC (i.e. < 10 mg COD/L).

Inhibitor entering with feed. To test the expert system against an inhibitor entering with feed, 40 g/L of phenol were added to the feed. Two tests were conducted; in the first test phenol was added to the digester for 12 hours and in the second test phenol was added for 36 hours.

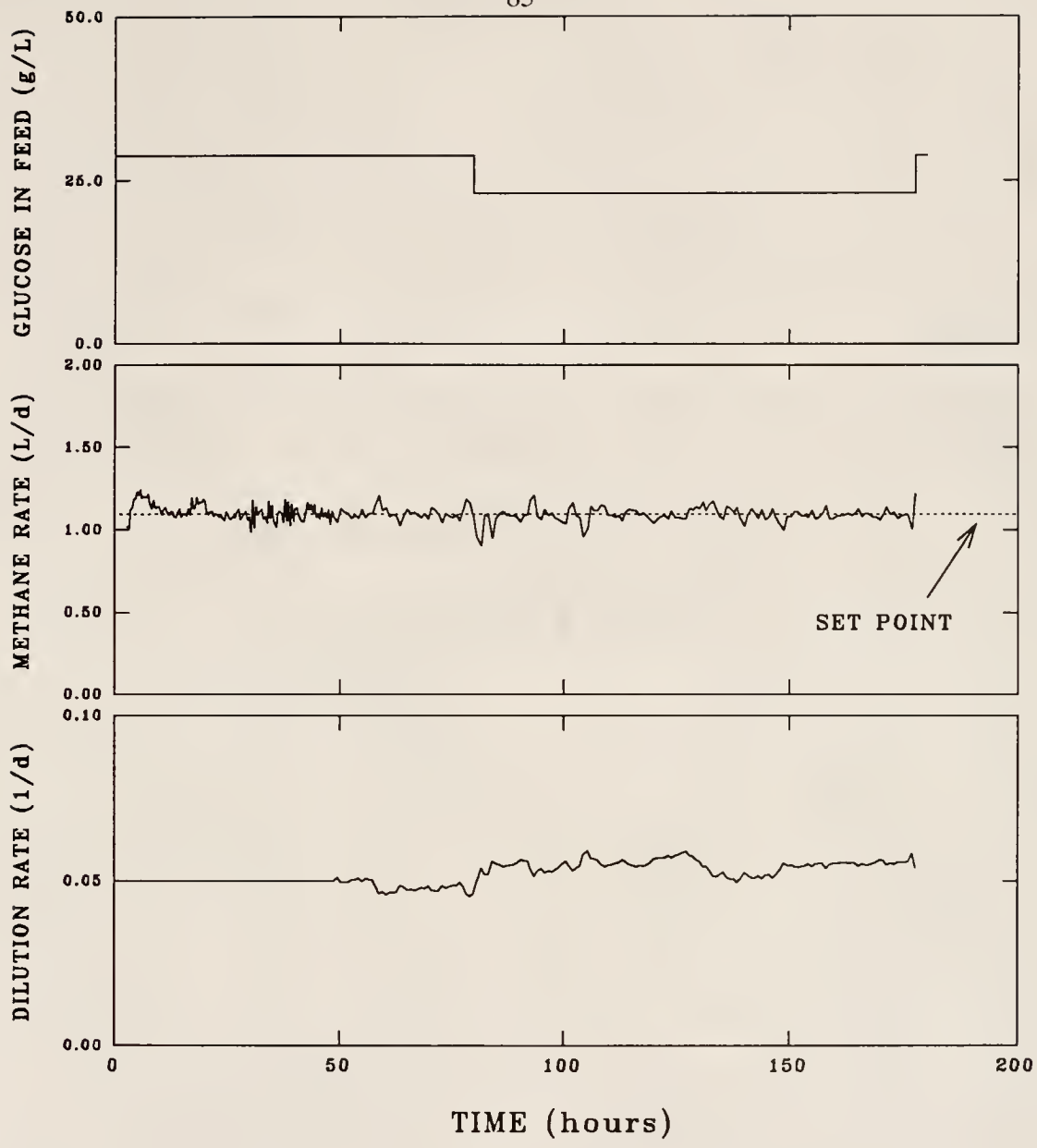


Figure 4-7: Experimental expert system response to a mild underload.

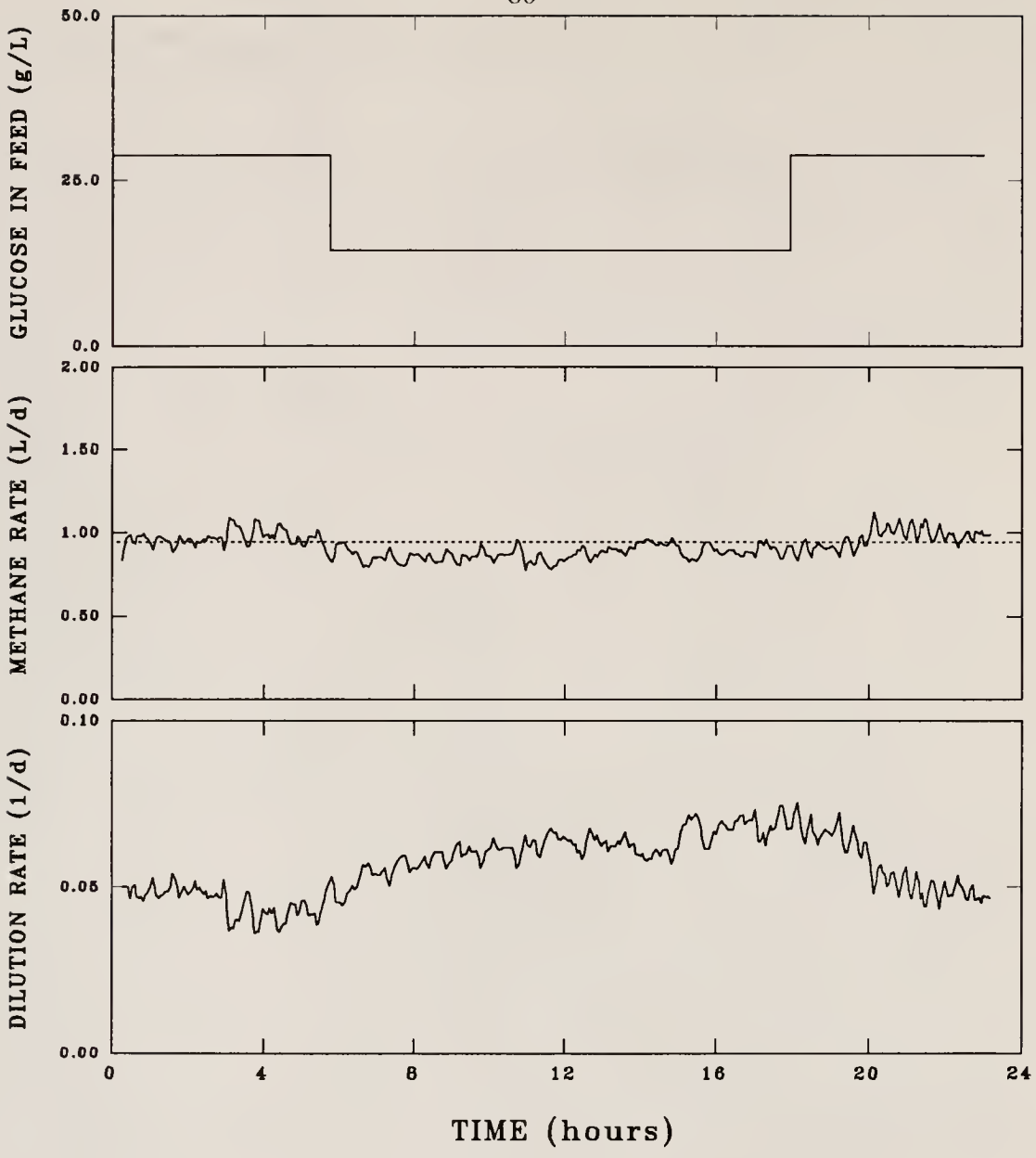


Figure 4-8: Experimental expert system response to a moderate underload.

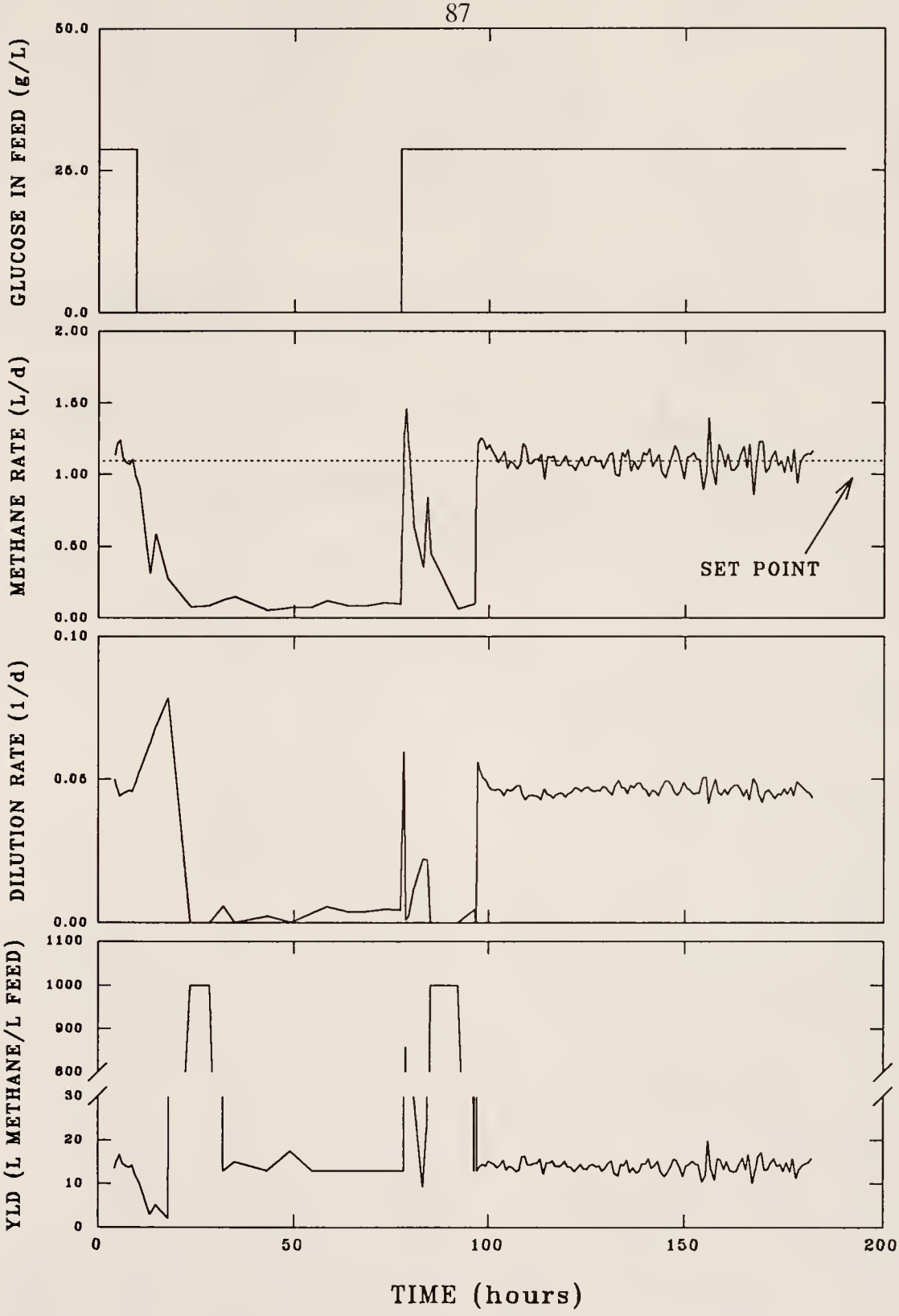


Figure 4-9: Experimental expert system response to a severe underload.

Figure 4-10 depicts the expert system response to a 12 hour phenol addition. The expert system was able to maintain set-point by increasing the dilution rate. This action did not cause any adverse effects on the microorganisms. Volatile organic acids began to accumulate; however they dropped after the phenol was removed. Phenol concentration in the digester reached 900 mg/L (Figure 4-11). This was not high enough a concentration to cause a reversal of the sign of the gain as a result the lowering of growth rates was compensated by an increase in dilution rate.

In the second test, after 27 hours of phenol addition the expert system sensed the onset of gain sign reversal. At this point the methane production rate had dropped below the lower bound and had continued to drop even after increasing the dilution rate (Figures 4-12). Subsequently, a batch phase followed. The methane production rate profile during batch operation is shown in Figure 4-12. When it was confirmed that the volatile organic acid concentration levels were not high the digester switched to the CYCL mode. This mode of operation continued until the methane reached rate the set point after which the mode of operation was switched to set point control. Figure 4-13 depicts the response of the expert system for the whole duration of the experiment. pH and individual volatile organic acid and phenol concentrations are shown in Figure 4-14.

During the constant yield operation the digester was operated at a retention time 40 days for most of the time. This indicates that there was a 50% inhibition of the process, because the digester could handle only half the nominal feed rate so as

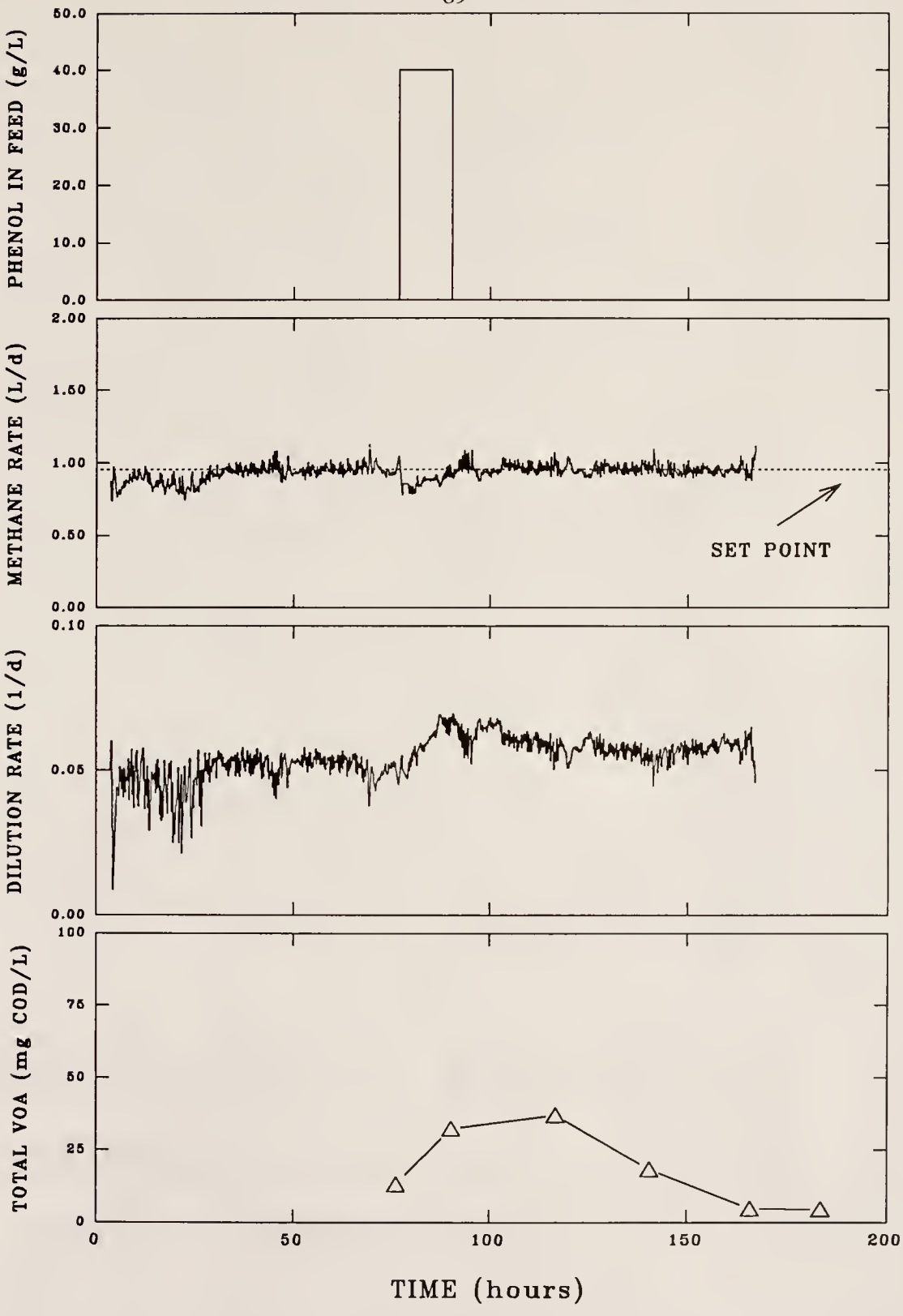


Figure 4-10: Experimental expert system response to a 12 hour phenol addition.

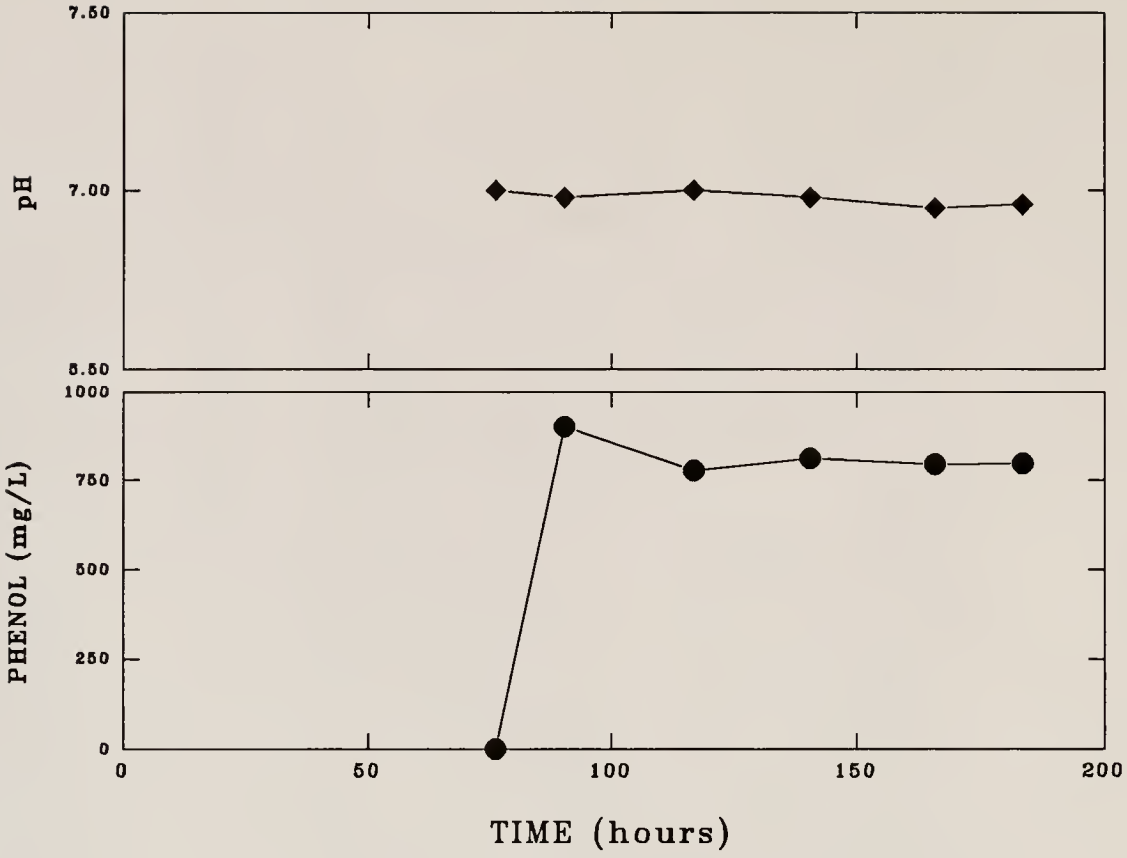


Figure 4-11: Experimental pH and phenol concentrations in the digester.

to maintain the nominal yield. This observation is consistent with that found in literature. Several investigators have studied phenol inhibition (Field, 1989) and have shown that about 2000 mg/L of phenol causes 50% inhibition. The concentration of phenol in the digester when it went batch was 1600 mg/L (Figure 4-14) which is close to the value reported in literature. Phenol was degraded into propionate through some intermediate compounds resulting in propionate accumulation in the digester (Figure 4-14). The presence of intermediate compounds is indicated by the fact that there was a delay between phenol depression and propionate accumulation (Figure 4-14). Propionate accumulated to levels of 4000 mg/L. Phenol degradation and the implications of using propionate as an indicator of digester imbalance are discussed in detail in the next chapter.

After the methane rate reached nominal set point the control was handed to the set point controller. Very soon after this the accumulated propionate started to degrade to acetate and then methane, causing an increase in the methane production rate. The controller immediately dropped the dilution rate assuming that this increase was due to an overload. Once all the propionate was degraded the dilution rate was brought back to 0.05 d^{-1}. Thus the expert system successfully operated the digester even under adverse conditions like high propionate and low pH.

Conclusions

An expert system to prevent digester imbalance was developed. The expert system uses on-line methane production rate measurements to decide the operating

strategy. The expert system was first simulated against the model presented in Chapter 2. It was successful in preventing imbalance when the process was subjected to a feed overload, feed underload and an inhibitor entering with the feed. The expert system was then implemented on a bench-scale digester. Disturbances in the form of feed overloads, feed underloads and phenol were tested. These disturbances, especially phenol, resulted in adverse growth conditions for the bacterial populations. However, the expert system effectively operated the digester irrespective of the type of disturbance.

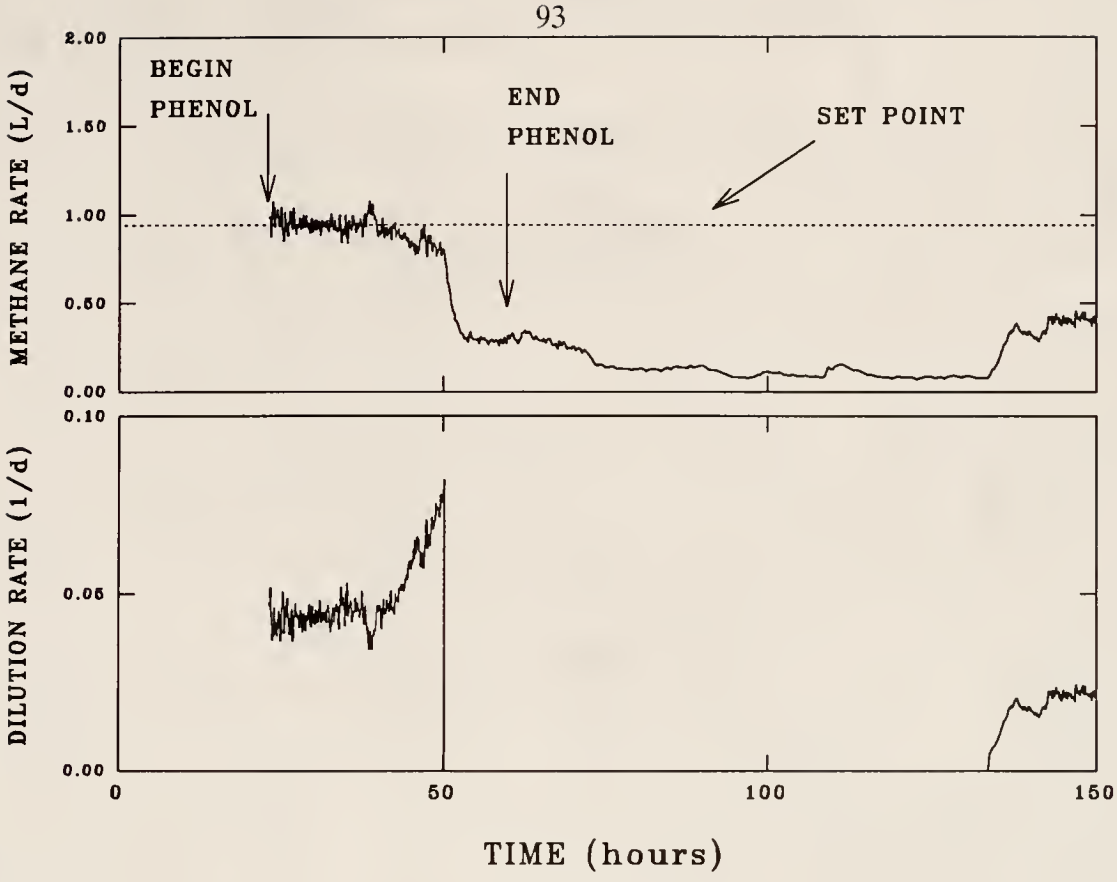


Figure 4-12: Experimental expert system response, showing reversal of steady state gain, to a 36 hour phenol addition.

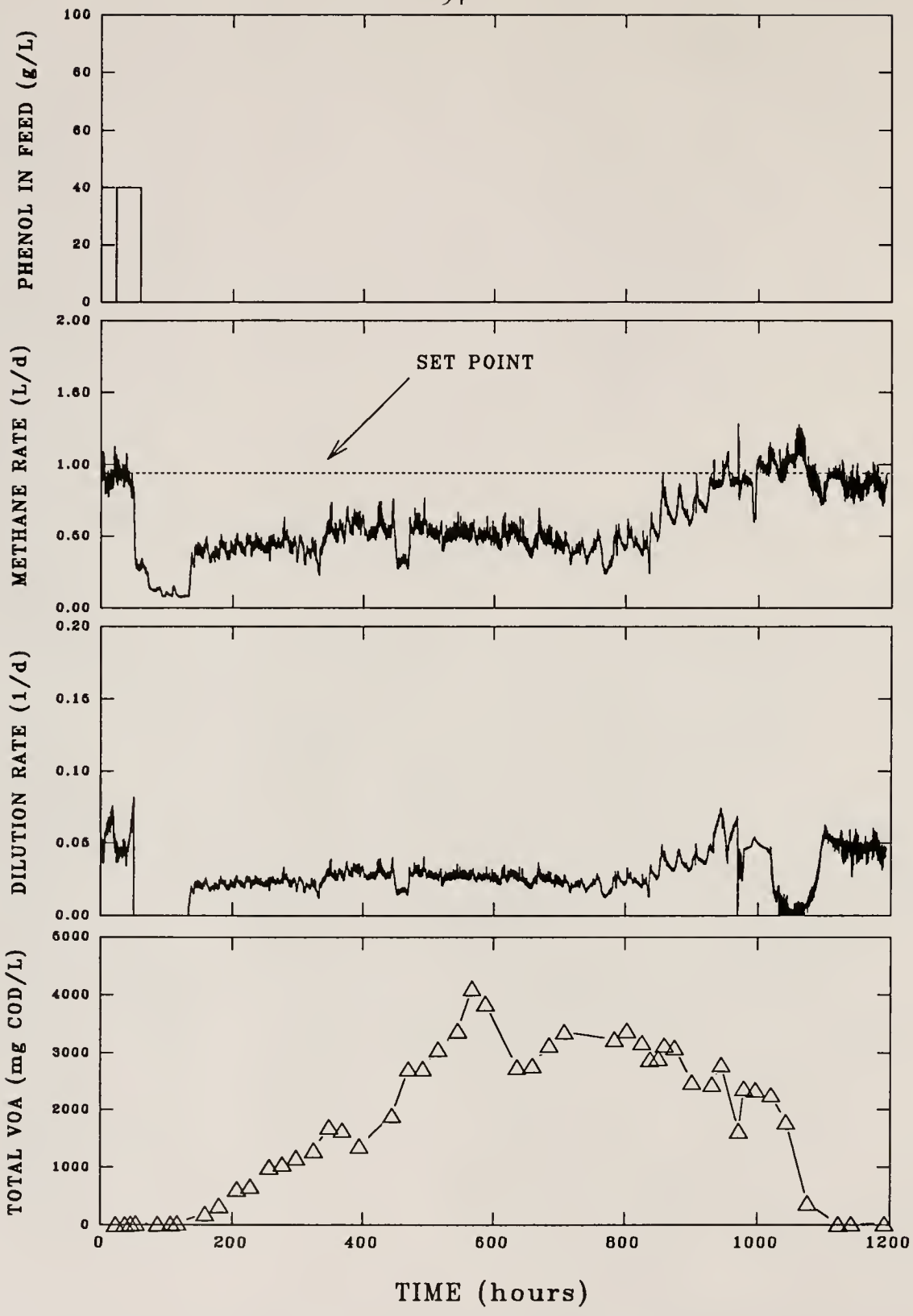


Figure 4-13: The complete expert system response to a 36 hour phenol addition.

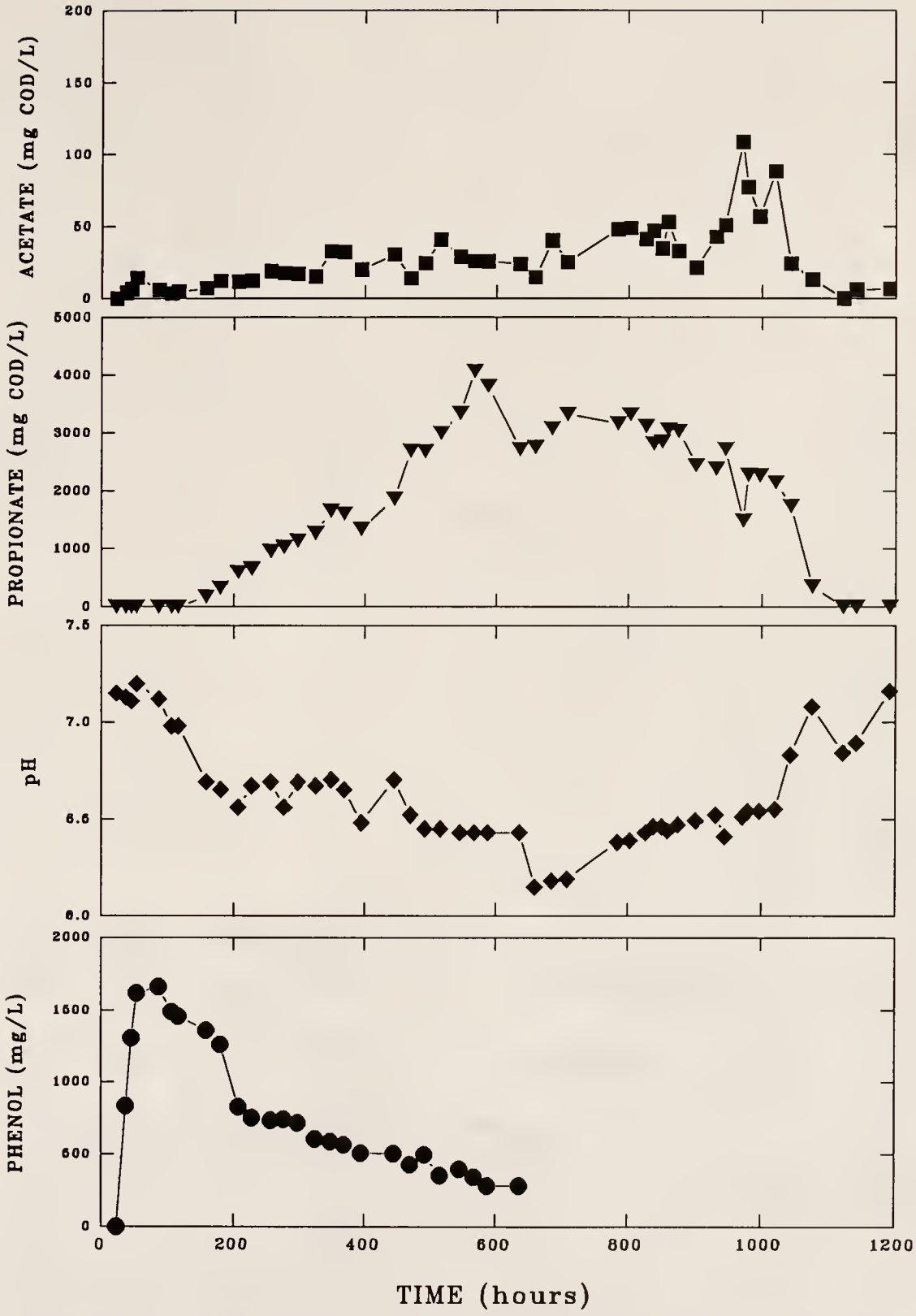


Figure 4-14: Experimental pH and individual volatile organic acid and phenol concentrations resulting from a 36 hour phenol addition.

CHAPTER 5
ON THE ASSESSMENT OF DIGESTER HEALTH USING VOLATILE ORGANIC ACID CONCENTRATION

During the operation of anaerobic digesters it was observed that, when the digester was imbalanced, the concentrations of volatile organic acids, especially propionic acid, in the digester was high. Presently, there are two schools of thought to explain the causes for the accumulation of volatile organic acids. One group of investigators (Andrews, 1969; Andrews and Graef, 1970; Hill *et al.*, 1983; Bryers, 1985; Smith *et al.*, 1988) believe that this buildup is due to the fact that when the methanogenic bacteria are inhibited, the volatile organic acids that are produced in the acidogenic step are no longer converted to methane. This is reflected in their approach to modeling the process. It is assumed that the acidogenic bacteria convert soluble organic material into acetic, propionic and butyric acid in a fixed ratio (Smith *et al.*, 1988). However, if this is true then when the digester is imbalanced then all three acids should accumulate at the same rate. But an imbalance of the process usually leads to only accumulation of propionic acid. It has been proposed by other investigators (Heyes and Hall, 1981; Mosey, 1982; Costello *et al.*, 1991a) that the major volatile organic acid produced during acidogenesis of carbohydrates is acetic acid and the higher organic acids are produced as a result of hydrogen accumulation during imbalance. This was incorporated into the model which was developed in

Chapter 2. Since concentrations of higher acids (propionic and butyric) are low under normal conditions, the digester does not contain a large population of propionate and butyrate utilization bacteria. Hence, when propionic and butyric acids accumulate these are not degraded easily, leading to build-up of acids. Both mechanisms indicate that the result of an imbalance is build-up of volatile organic acids.

Numerous observations related to anaerobic digestion of wastes suggest that volatile organic acid relationships have a direct correlation with digester performance. Propionic acid levels have been found to rise prior to failure of digesters treating swine wastes (Fischer *et al.*, 1981), beef wastes (Hashimoto *et al.*, 1978), municipal sludge (Kaspar and Wuhrmann, 1978) and food processing wastes (van den Berg and Lentz, 1977). Nordstedt and Thomas (1985) suggested using the propionic to acetic acid ratio as an indicator of digester performance. Hill *et al.*(1987) conducted an extensive survey of the literature for digester performance data and volatile organic acid levels. They used 70 observations to arrive at a relationship between acetic level, propionic to acetic acid ratio and digester performance. They proposed that acetic acid levels in excess of 800 mg/L or a propionic to acetic acid ratio greater than 1.4 indicated impending digester failure.

The issue is the reliability in using volatile organic acid measurements for purposes of predicting digester failure. The phenol inhibition experiment, reported in the last chapter, resulted in propionic acid concentrations of 4000 mg COD/L (or 2643 mg/L) and even at this concentration the digester continued to produce

methane (Figures 4-13 and 4-14). During this period the acetic acid concentration was 50 mg COD/L (or 47 mg/L). The ratio of propionic to acetic acid concentration is 56. This ratio is much greater than the value of 1.4 suggested by Hill *et al.* (1987). To use propionic acid levels as an indicator of digester instability it is necessary to determine the reactions leading to formation of the propionic acid.

In the following discussion it is argued that the accumulation of propionic acid was the result of the degradation of the phenol and was not due to hydrogen build up. Figure 5-1 compares the actual phenol concentration in the digester (points) to simulated digester phenol concentrations assuming no degradation of phenol (solid lines 1 & 2) for the phenol inhibition experiment shown in Figures 4-13 and 4-14. The simulated values were obtained from the equation

$$I_2 = I_1 e^{-D(t_2-t_1)}$$

where I_2 = phenol concentration in the digester at sampling time t_2

I_1 = phenol concentration in the digester at sampling time t_1

D = dilution rate set by the controller during the sampling interval $(t_2 - t_1)$ (Figure 4-8)

Vapor phase phenol that escapes through the vented gas was neglected. Two simulations were performed. The first simulation (solid line 1 in Figure 5-1) calculated phenol concentrations in the digester starting with an initial concentration of 1662.0 mg/L, the maximum experimental phenol concentration. The second simulation (solid line 2 in Figure 5-1) calculated phenol concentration in the digester

starting with an initial concentration of 670.3 mg/L, the experimental phenol concentration at 200 hours.

Comparison of the solid line 1 to experimental phenol concentrations definitly showed evidence for phenol degradation. Simulated phenol concentration remained constant during an initial batch phase, however the actual phenol levels started dropping immediately. This drop continued until time 200 hours, when the concentration dropped to 670.3 mg/L. This discrepancy indicated the possibility that phenol was being degraded in the digester. Phenol degradation by anaerobic bacteria has been reported in Healy and Young (1978, 1979), Young and Rivera (1985), Knoll and Winter (1987, 1989). Young and Rivera (1985) suggested the following mechanism for the degradation of phenol (Figure 5-2). The phenol ring is saturated yielding cyclohexanol and cyclohexanone. The cyclohexanone then undergoes a non-oxygenase mediated ring fission resulting in the formation of adipic acid or caproic acid. These compounds are degraded to organic acid metabolites which are then metabolized to the end products carbon dioxide and methane. This mechanism could reasonably explain the accumulation of propionic acid (Figure 4-14). Propionic acid did not start accumulating until after 200 hours by which time the phenol had dropped by 700 mg/L. This delay can be attributed to the formation of intermediates cyclohexanol, cyclohexanone adipic acid and caproic acid. The propionic acid could not have been formed from the glucose that was fed because the digester was operated in a constant yield mode by keeping the ratio of glucose fed to methane produced constant. If the glucose were to be converted to propionic

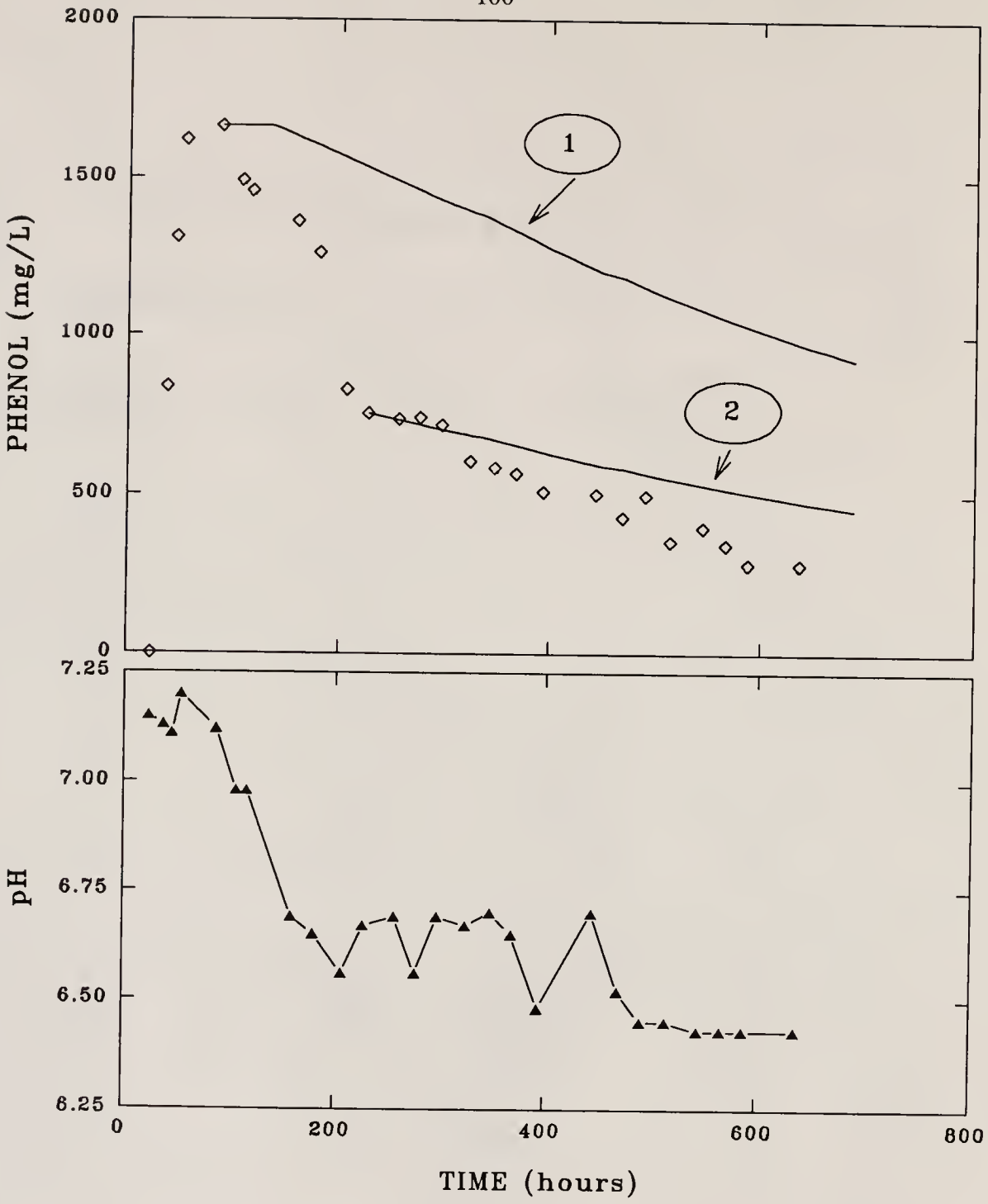


Figure 5-1: Comparison of actual phenol concentrations in the digester (points) to simulated concentrations (solid lines) assuming no phenol degradation.

acid then the methane production rate would have dropped, resulting in a proportional drop in dilution rate. Instead, the dilution rate was maintained around 0.025 d_{-1} for a period of 650 hours. Hence it was concluded that the phenol was degraded to propionate. However, after 200 hours there is very little degradation of phenol. This was demonstrated by comparing solid line 2 in Figure 5-1 to experimental data. By 200 hours the pH in the digester had dropped to 6.6 (Figure 5-1), the same time that phenol-degradation ceased. This indicated that phenol degradation was pH dependent.

Nevertheless, it can be argued that the methanogenic population was at least 50% inhibited, otherwise the constant yield control law would have brought the dilution rate back to 0.5 d^{-1} immediately. This inhibition was possibly due to the phenol and not propionic acid because even before propionic acid started to accumulate the constant yield control law operated the digester at a dilution rate of 0.025 d^{-1}.

The above observations indicated that using propionic acid accumulation as an indicator of digester imbalance is unreliable. In digesters treating wastes, the composition of the feed media may not be known and may contain different substrates. It is possible that anaerobic bacteria could degrade any number of these substrates to propionic acid leading to unusual build up of propionic acid in the digester. If volatile organic acid levels are used as indicators for digester imbalance then this build-up would definitely lead the operator into erroneous conclusions about digester performance. However, if this propionic acid accumulation was due

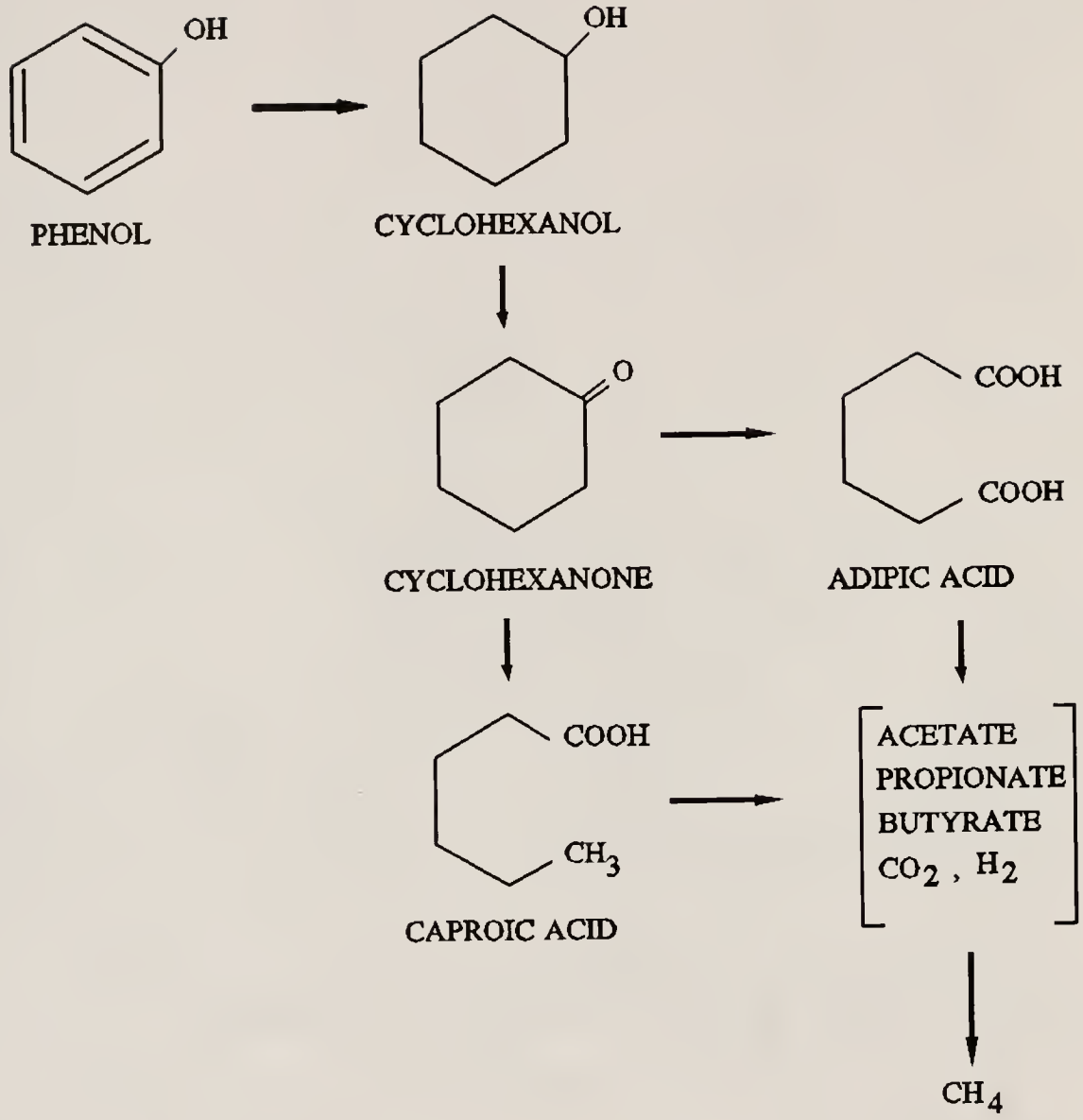


Figure 5-2: Degradation of phenol by anaerobic bacteria.

to hydrogen build-up, then the levels of 2500 mg/L of propionic acid in digester would definitely indicate failure. Hence, it is important to know the reactions leading to the formation of propionate.

CHAPTER 6
CONCLUSIONS

The major objective of this work was to develop an on-line automatic control strategy to prevent and mitigate imbalance in anaerobic digesters. This was achieved by developing an expert system that based its decisions on on-line methane production rate measurements. The expert system was implemented on a bench scale digester and was shown to successfully prevent imbalance of the anaerobic digestion process, when the process was exposed to feed overloads, feed underloads and inhibitors entering with feed was developed. However, during the course of this study a few other projects were accomplished:

a) A dynamic model that predicts the behavior of the digester when it is subjected to a feed overload was developed. The model incorporates the most recent knowledge of the process and is also able to calculate important design parameters like gas production rates, gas composition and pH. The model was used as a simulator for the digestion process during the development of the expert system.

b) Until now the industry did not have a control policy that could moderate the effect of an inhibitor as it enters the digester through the feed. This need has been fulfilled by developing a control strategy using a simplified model for the process and Pontryagin's maximum principle. It was shown that the optimal control

strategy can be replaced by a constant yield control policy without any loss of performance. The constant yield control policy changes the dilution rate in proportion to the methane production rate. It should be noted that this policy can be used for other biochemical processes where instead of methane production rate, the rate of production of the desired product should be used.

c) Contrary to popular practice it was found that volatile organic acid concentrations and pH levels cannot be used reliably to predict digester imbalance. The microbial consortia was able to tolerate propionic acid concentrations of 3000 mg/L and pH levels of 6.1.

There is still room for improvement. It is possible to make the expert system more robust and more sensitive by incorporating other measurements like NADH fluorescence and gas composition. NADH fluorescence measurements can enable the controller to distinguish between and an underload and an inhibitor very soon after the inhibitor enters the digester, thus enabling the controller to take early corrective actions. It is believed that in response to an inhibitor methane percent in the gas phase drops immediately and hence gas composition measurements would also enable early corrective action. However, studies need to be done to determine the viability of using gas composition measurements to identify feed overloads, feed underloads or inhibitors in feed. Gas composition is easily measured on industrial scale digesters and a controller based on gas composition can be readily implemented.

APPENDIX A
DYNAMIC MODEL FOR A GLUCOSE FED CONTINUOUSLY STIRRED ANAEROBIC DIGESTER

Nomenclature

$[\ ]$ = concentration in liquid phase

d/dt = rate of accumulation

Y = yield coefficient

r = rate of formation

p = partial pressure in the gas phase

Q = gas production rate

μ = specific growth rate

μ_{max} = maximum specific growth rate

k_G = maximum rate constant for acidogenic bacteria

K_s = Monod half velocity constant

KIH = inhibition due to hydrogen

K_e = equivalent dissociation constant of volatile organic acids

K_{bc} = dissociation constant for $(CO_2)_D$ - HCO_3^- system

KL_a = overall mass transfer coefficient for CO_2

K_{HL} = Henry's law constant

T_g = dissolved carbon dioxide gas transfer rate

R_{id} = liters of gas per mole, assuming ideal gas behaviour

V = volume of the liquid phase

V_g = volume of the gas phase

P_T = total pressure of the gas phase

D = dilution rate ($days^{-1}$)

Xa = acidogenic bacteria

Xp = propionate utilizing acetogenic bacteria

Xb = butyrate utilizing acetogenic bacteria

Xm = acetoclastic methane bacteria

Xh = hydrogen utilizing methane bacteria

PYR = pyruvate

G = glucose

A = acetate

P = propionate

B = butyrate

CO_2 = carbon dioxide

$(CO_2)_D$ = carbon dioxide dissolved in the liquid phase

HCO_3^- = bicarbonate ion

TC = total inorganic carbon

H^+ = hydrogen ion

Z = net cations

H_2 = hydrogen

CH_4 = methane

NADH = reduced form of the coenzyme nicotinamide adenine dinucleotide

NAD^+ = oxidized form of the coenzyme nicotinamide adenine dinucleotide

Superscripts

Ad = acidogenesis step

At = acetogenesis step

M = methanogenesis from acetate step

H = hydrogen utilizing step

s = synthesis of microorganisms step

Subscripts

o = feed concentration

gas = total gas i.e., sum of methane, carbon dioxide and hydrogen

PYR/G = pyruvate from glucose

A/G = acetate from glucose

P/G = propionate from glucose

B/G = butyrate from glucose

Xa/A = acidogens from acetate

Xa/P = acidogens from propionate

Xa/B = acidogens from butyrate

Xa/G = acidogens from glucose

A/P = acetate from propionate

A/B = acetate from butyrate

Xp/P = propionate utilizing acetogens from propionate

Xb/B = butyrate utilizing acetogens from butyrate

Xm/A = acetoclastic methanogens from acetate

Xh/H_2 = hydrogen utilizing methanogens from hydrogen

CO_2/A = carbon dioxide produced per unit acetate produced

CO_2/B = carbon dioxide produced per unit butyrate produced

CO_2/P = carbon dioxide produced per unit propionate produced

CO_2/Xp = carbon dioxide produced (or consumed) per unit propionate utilizers produced

CO_2/Xb = carbon dioxide produced (or consumed) per unit butyrate utilizers produced

CO_2/H_2 = carbon dioxide consumed per unit hydrogen consumed

CO_2/Xh = cardon dioxide consumed per unit hydrogen utilizers produced

H_2/A = hydrogen produced per unit acetate produced

H_2/P = hydrogen from propionate

H_2/B = hydrogen produced per unit butyrate produced (or consumed)

H_2/Xp = hydrogen produced per unit propionate utilizer produced

CH_4/A = methane from acetate

CH_4/H_2 = methane from hydrogen

Dynamic Model

Rates of consumption of glucose and pyruvate

$$r'_G = \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)} \frac{k_G\,[G]}{K_{s,G} + [G]}\,[Xa]$$

$$r'_{PYR} = \frac{\alpha\,[PYR]}{K_{s,PYR} + [PYR]}\,[Xa]$$

Rates of formation of volatile organic acids from glucose

$$r_{P/G} = \frac{1}{\left(1 + \frac{[NAD^+]}{[NADH]}\right)} r'_{PYR}$$

$$r_{B/G} = \frac{1}{2} \frac{1}{\left(1 + \frac{[NAD^+]}{[NADH]}\right)} \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)} r'_{PYR}$$

$$r_{A/G} = \frac{1}{\left(1 + \frac{[NADH]}{[NAD^+]}\right)^2} r'_{PYR}$$

Specific growth rates

$$\mu_{Xp} = \frac{\mu_{max,Xp}\ [P]}{(K_{s,P} + [P])\ (1 + KIH_{Xp} p_{H_2})}$$

$$\mu_{Xb} = \frac{\mu_{max,Xb}\ [B]}{(K_{s,B} + [B])\ (1 + KIH_{Xb} p_{H_2})}$$

$$\mu_{Xm} = \frac{\mu_{max,Xm}\ [A]}{([A] + K_{s,A})\ (1 + KIH_{Xm} p_{H_2})}$$

$$\mu_{Xh} = \frac{\mu_{max,Xh}\ p_{H_2}}{(K_{s,Xh} + p_{H_2})}$$

where

$$\mu_{max,Xh} = \frac{31.93}{\left(1 + \frac{[H^+]}{1.273 \times 10^{-8}} + \frac{7.58 \times 10^{-7}}{[H^+]}\right)}$$

Net rates of formation

$$r_G = -r'_G - \frac{1}{Y^{Ad,s}_{XA/G}} \left(Y^{Ad}_{Xa/A} r_{A/G} + Y^{Ad}_{Xa/P} r_{P/G} + Y^{Ad}_{Xa/B} r_{B/G}\right)$$

$$r_{PYR} = 2r'_G - r'_{PYR}$$

$$r_A = r_{A/G} + \frac{Y^{At}_{A/P}}{Y^{At}_{Xp/P}} \mu_{Xp}[Xp] + \frac{Y^{At}_{A/B}}{Y^{At}_{Xb/B}} \mu_{Xb}[Xb] - \frac{1}{Y^{M}_{Xm/A}} \mu_{Xm}[Xm] - \frac{1}{Y^{M,s}_{Xm/A}} \mu_{Xm}[Xm]$$

$$r_P = r_{P/G} - \frac{1}{Y^{At}_{Xp/P}} \mu_{Xp}[Xp] - \frac{1}{Y^{At,s}_{Xp/P}} \mu_{Xp}[Xp]$$

$$r_B = r_{B/G} - \frac{1}{Y^{At}_{Xb/B}} \mu_{Xb}[Xb] - \frac{1}{Y^{At,s}_{Xb/B}} \mu_{Xb}[Xb]$$

$$r_{H_2} = Y^{Ad}_{H_2/A} r_{A/G} - Y^{Ad}_{H_2/P} r_{P/G} + Y^{Ad}_{H_2/B} r_{B/G} + \frac{Y^{At}_{H_2/P}}{Y^{At}_{Xp/P}} \mu_{Xp} [Xp]$$

$$+ \frac{Y^{At}_{H_2/B}}{Y^{At}_{Xb/B}} \mu_{Xb} [Xb] - \frac{1}{Y^{H}_{Xh/H_2}} \mu_{Xh} [Xh]$$

$$+ \frac{1}{Y^{At,s}_{Xp/H_2}} \mu_{Xp} [Xp] - \frac{1}{Y^{H,s}_{Xh/H_2}} \mu_{Xh} [Xh]$$

$$r_{CO_2} = Y^{Ad}_{CO_2/A} r_{A/G} + Y^{Ad}_{CO_2/B} r_{B/G} + \frac{Y^{At}_{CO_2/P}}{Y^{At}_{Xp/P}} \mu_{Xp} [Xp] + \frac{Y^{M}_{CO_2/A}}{Y^{M}_{Xm/A}} \mu_{Xm} [Xm]$$

$$- \frac{Y^{H}_{CO_2/H_2}}{Y^{H}_{Xh_{H_2}}} \mu_{Xh} [Xh] - Y^{At,s}_{CO_2/Xp} \mu_{Xp} [Xp]$$

$$- Y^{At,s}_{CO_2/Xb} \mu_{Xb} [Xb] - Y^{H,s}_{CO_2/Xh} \mu_{Xh} [Xh]$$

$$r_{CH_4} = \frac{Y^{M}_{CH_4/A}}{Y^{M}_{Xm/A}} \mu_{Xm} [Xm] + \frac{Y^{H}_{CH_4/H_2}}{Y^{H}_{Xh/H_2}} \mu_{Xh} [Xh]$$

$$r_Z = -Y_Z \left(Y^{Ad}_{Xa/A} r_{A/G} + Y^{Ad}_{Xa/P} r_{P/G} + Y^{Ad}_{Xa/B} r_{B/G} + \mu_{Xp} [Xp] \right.$$

$$\left. + \mu_{Xb} [Xb] + \mu_{Xm} [Xm] + \mu_{Xh} [Xh] \right)$$

Physico-chemical relationships

$$[HCO_3^-] = \frac{K_{bc}[TC]}{(K_{bc}+[H^+])}$$

$$[Z] + [H^+] = \frac{K_e}{(K_e+[H^+])}([A]+[P]+[B]) + K_{bc}\frac{[TC]}{(K_{bc}+[H^+])}$$

$$[(CO_2)_D] = [TC] - [HCO_3^-]$$

$$T_g = KL_a(K_{HL}p_{CO_2} - [(CO_2)_D])$$

$$\frac{[NADH]}{[NAD^+]} = 10^{(\log p_{H_2} - \log[H^+] - 3.824)}$$

Gas production rates

$$Q_{CO_2} = -T_g\hat{V}_{id}V$$

$$Q_{H_2} = r_{H_2} \hat{V}_{id} V$$

$$Q_{CH_4} = r_{CH_4} \hat{V}_{id} V$$

$$Q_{gas} = Q_{CO_2} + Q_{CH_4} + Q_{H_2}$$

State equations

$$\frac{d[G]}{dt} = D([G_{in}] - [G]) + r_G$$

$$\frac{d[Xa]}{dt} = -D[Xa] + Y^{Ad}_{Xa/A} r_{A/G} + Y^{Ad}_{Xa/P} r_{P/G} + Y^{Ad}_{Xa/B} r_{B/G}$$

$$\frac{d[PYR]}{dt} = -D[PYR] + r_{PYR}$$

$$\frac{d[A]}{dt} = -D[A] + r_A$$

$$\frac{d[P]}{dt} = -D[P] + r_p$$

$$\frac{d[B]}{dt} = -D[B] + r_B$$

$$\frac{d[Xp]}{dt} = -D[Xp] + \mu_{Xp}[Xp]$$

$$\frac{d[Xb]}{dt} = -D[Xb] + \mu_{Xb}[Xb]$$

$$\frac{d[Xm]}{dt} = -D[Xm] + \mu_{Xm}[Xm]$$

$$\frac{d[Xh]}{dt} = -D[Xh] + \mu_{Xh}[Xh]$$

$$\frac{d[TC]}{dt} = D([TC_{in}] - [TC]) + r_{CO_2} + T_g$$

$$\frac{d[Z]}{dt} = D([Z_{in}] - [Z]) + r_Z$$

$$\frac{dp_{CO_2}}{dt} = \frac{P_T Q_{CO_2}}{V_g} - \frac{p_{CO_2} Q_{gas}}{V_g}$$

$$\frac{dp_{H_2}}{dt} = \frac{P_T Q_{H_2}}{V_g} - \frac{p_{H_2} Q_{gas}}{V_g}$$

$$\frac{dp_{CH_4}}{dt} = \frac{P_T Q_{CH_4}}{V_g} - \frac{p_{CH_4} Q_{gas}}{V_g}$$

APPENDIX B
FEED PREPARATION

Glucose and feed media was sterilized and refrigerated during digester operation. The normal glucose media was prepared using the ingredients outlined in Table B-1. The trace nutrient mineral solution used is outlined in Table B-2. The feed media was prepared by dissolving all ingredients less the mineral solution and diammonium phosphate in a 2-L polypropylene flask while sparging with nitrogen gas. The feed flask was then capped with a stopper fashioned with 3 glass tubes, one of which reaches to the bottom of the flask. After autoclaving at 20 psi for 10 minutes, a tedlar gas bag filled with nitrogen gas was connected to one of the inlet tubes while the solution cooled. After cooling, the mineral solution and diammonium phosphate solution were added via sterile syringe.

Table B-1
Standard glucose media used in digester under normal operation

Reagent	Amount
Glucose (dextrose)	28.8 g
Yeast extract (Difco)	3.2 g
Casamino acids (Difco)	3.2 g
$NaHCO_3$	3.0 g
$NaCH_3CH_2COOH$	1.0 g
Mineral solution, S4	15.0 mL
$(NH_4)_2HPO_4$ solution (26.7 g L^{-1})	5.0 mL
Distilled water to	1.0 L

Table B-2
Mineral solution, S4

Compound	Concentration (g L^{-1})
$CaCl_2 \cdot 2H_2O$	16.7
NH_4Cl	26.6
$MgCL_2 \cdot 6H_2O$	120
KCl	86.7
$MnCl_2 \cdot 4H_2O$	1.33
$CoCl_2 \cdot 6H_2O$	2.0
H_3BO_3	0.38
$CuCl_2 \cdot 2H_2O$	0.18
$Na_2MoO_4 \cdot 2H_2O$	0.17
$ZnCl_2$	0.14
$NiCl_2 \cdot 6H_2O$	0.15
H_2WO_4	0.007

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